Enalapril and the kidney : clinical studies of the renal effects of enalapril in antihypertensive treatment

Navis, Gerarda Janna

Gerjan Navis

ENALAPRIL AND THE KIDNEY

Clinical studies of the renal effects of enalapril in antihypertensive treatment

STELLINGEN.

1. ACE-remmers zijn (ook) diuretica.
2. Zoutbeperking potentieert de bloeddrukverlagende werking van ACE-remmers. Uit oogpunt van fysiologie is het juister te stellen, dat ACE-remmers de bloeddrukverlagende werking van zoutbeperking potentieren.
3. ACE-remming vergroot de drop-gevoeligheid van de bloeddruk.
4. Er zijn goede argumenten om aan te nemen dat behandeling met ACE-remmers bij patienten met gestoorde nierfunktie nierfunktie-sparend zal werken.
5. Aan de rash, optredend bij gebruik van captopril is noch ACE-remming noch de sulfhydrylgroep debet.
6. Met de geslachtsgebonden verschillen in cardiovasculair risico wordt in de door de Gezondheidsraad aanbevolen behandelingsstrategie voor te hoge bloeddruk ten onrechte geen rekening gehouden.
7. Uit gezondheidsoogpunt dient de appetijtelijkheid van ziekenhuisvoeding maximaal te zijn.
8. De multi-racialiteit van de Nederlandse samenleving maakt ook de geneeskunde kleuriger.
9. Ook voor artsen zou de arbeidswet moeten gelden.
10. De uitspraak van de Raad van State dat het Fries in Friesland geen bestuurstaal kan zijn, ontkent de maatschappelijke realiteit.
11. Gelet op de inhoud en hoeveelheid van het aanbod van oude en nieuwe media lijkt het non-informatie-tijdperk te zijn aangebroken.

Stellingen
behorende bij het proefschrift van
Gerjan Navis
Groningen
24 september 1986

ENALAPRIL AND THE KIDNEY

CLINICAL STUDIES OF THE RENAL EFFECTS OF ENALAPRIL IN ANTIHYPERTENSIVE TREATMENT

to my parents
to Auke

RIJKSUNIVERSITEIT TE GRONINGEN

Enalapril and the Kidney

CLINICAL STUDIES OF THE RENAL EFFECTS OF ENALAPRIL IN ANTIHYPERTENSIVE TREATMENT

Proefschrift

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op
woensdag 24 september 1986 des namiddags te 4.00 uur

door

GERARDA JANNA NAVIS

geboren te Groningen

1986

DRUKKERIJ
HOOGCARSPEL
BEEMSTER

promotores: prof.dr. G.K. van der Hem
prof.dr. A.J.M. Donker
referents: dr. P.E. de Jong
dr. D. de Zeeuw

ISBN 90-9001384-9

Parts of the studies described here, and the printing of this thesis were made possible by financial support of MSD. The renal function studies were made possible by Grants from the Nier Stichting Nederland.

CONTENTS

ACKNOWLEDGEMENTS.

The studies resulting in this thesis were performed during the years 1982-1985 in the Department of Nephrology (Head Prof. Dr. G.K van der Hem) and the Outpatient Clinic for Hypertension (Head Dr B.S ten Berge), both part of the Department of Internal Medicine (Head Prof. Dr. E Mandema) of the State University Hospital Groningen.

During those years many people have contributed to my scientific and personal education as well as to the actual realization of the studies. To the following persons in particular I wish to express my gratitude. Prof. Dr. G.K van der Hem for his continuous interest and support in scientific and in personal matters. Prof. Dr. A.J.M Donker for his always stimulating and creative comments. Dr. Paul de Jong and Dr. Dick de Zeeuw, who were the initiators and stimulators of the studies, for introducing me into scientific work, for their refreshing discussions and for their never-ceasing enthusiastic support. Mrs Nel Hesling-Kuiper for her energetic dedication that made the work in the Outpatient Clinic a pleasure. Mrs Aly Drent-Bremer for the cheerful way in which she did the numerous renal function studies. The enthusiastic cooperation of the staff of dieticians was essential to all the studies. Without the support of Mr van Eekeren and Mr J Ritsema the sodium balance studies would have ended in chaos. The figures in this thesis were patiently drawn and re-drawn by Mr Jan Brouwer. Mr Bert Schaalma was always helpful in providing litterature. Mrs Greet Smit assisted in the preparation of the present and other manuscripts. The nursing staff of the wards B2, A2 and B3 took care of the patients in the in-patient studies. It is a pleasure to recall the cooperation of the patients participating in the sometimes exhausting studies; I am indebted to them for their patience and their sometimes remarkable self-observations. Finally I want to express my gratitude to my parents for providing the background that made it possible for me to prepare this thesis and enjoy it, and to Auke, for making it all worthwile.

LIST OF ABBREVIATIONS.

AI	: Angiotensin I.
AII	: Angiotensin II.
ACE	: Angiotensin I-converting enzyme.
BW	: Body weight
ERPF	: Effective renal plasma flow.
FF	: Filtration fraction.
GFR	: Glomerular filtration rate.
MAP	: Mean arterial pressure.
PAC	: Plasma aldosterone concentration.
PRA	: Plasma renin activity.
RAAS	: Renin-angiotensin-aldosterone system.
UKV	: urinary excretion of potassium.
UNaV	: urinary excretion of sodium.

PUBLICATIONS

Navis GJ, de Jong PE, Donker AJM, de Zeeuw D (1983).
Effects of enalapril on blood pressure and renal haemodynamics in essential hypertension. *Proc EDTA* 20; 577-581.

de Zeeuw D, Navis GJ, Donker AJM, de Jong PE (1983).
The angiotensin converting enzyme inhibitor enalapril and its effects on renal function. *J. Hypertens* 1 (suppl); 93-97.

Navis GJ, de Jong PE, Kallenberg CGM, de Monchy JGR, de Zeeuw D (1984).
Absence of cross-reactivity between captopril and enalapril. *Lancet i*; 1017.

Navis GJ, de Jong PE, Donker AJM, van der Hem GK, de Zeeuw D (1985).
Blood pressure response to enalaprilic acid; dose-response and effect of pre-treatment with furosemide. *Eur J Clin Pharmacol* 29; 9-15.

Navis GJ, de Jong PE, Donker AJM, van der Hem GK, de Zeeuw D (1985).
Effects of enalaprilic acid on sodium excretion and renal hemodynamics in essential hypertension. *J. Clin Hypertens* 1; 228-238.

Navis GJ, de Jong PE, de Zeeuw D (1985).
Serum uric acid, ACE-inhibitors and natriuresis. *Lancet ii*; 156.

Navis GJ, de Jong PE, Donker AJM, van der Hem GK, de Zeeuw D (1985).
Enalapril and the kidney: renal vasodilation and natriuresis due to the inhibition of angiotensin II formation. *J. Cardiovasc Pharmacol*, 8 (supp 1): S30-S34.

Navis GJ, de Jong PE, Donker AJM, van der Hem GK, de Zeeuw D.
Diuretic effects of ACE-inhibition; comparison of low and liberal sodium diet in hypertensive patients. *J. Cardiovasc Pharmacol*; in press.

Navis GJ, de Jong PE, Donker AJM, van der Hem GK, de Zeeuw D Renal response to moderate sodium restriction in essential hypertension; effects of ACE-inhibition. Submitted.

de Zeeuw D, Navis GJ, Donker AJM, van der Hem GK, de Jong PE.
Natriuretic effect of the angiotensin converting enzyme (ACE) inhibitor enalapril in patients with essential hypertension. *Proc 2nd Int Conf on Diuretics)* in press.

CHAPTER 1 INTRODUCTION.

Introduction.

The effects of enalapril in the treatment of essential hypertension with special emphasis on the renal effects, form the subject of this study. Enalapril is a recently introduced antihypertensive drug, being the second representative of a generation of orally active angiotensin converting enzyme (ACE)inhibitors of whom captopril was the first. The concept of ACE-inhibition constitutes probably one of the major recent advances in both our understanding and the management of high blood pressure to date. ACE-inhibitors are the first generation of antihypertensives that is the result of purposeful drug design, as they were specifically developed to interfere with the RAAS (Ondetti 1977).

Renin had since long been suspected to play a role in the pathophysiology of hypertension, especially in patients with malignant hypertension associated with renal and/or renovascular disease. Indeed captopril proved to be highly effective in this category of patients (Atkinson 1980, Bravo 1979), in general characterized by high levels of circulating renin. Unexpectedly, captopril was also effective in patients with milder forms of hypertension including patients with low levels of circulating renin (Veterans Administration Cooperative Study Group 1983, Vidt 1982). This finding has aroused new questions as to the pathophysiology of hypertension and the mechanism of action of ACE-inhibitors, some of which will be dealt with in this thesis. From the clinical point of view, this finding may even lead to wide spread application of ACE-inhibitors as first-line drugs in the management of hypertension, and to an alternative to the traditional stepped-care approach (Tarazi 1985, Zanchetti 1985).

In a study dealing with a new first-line antihypertensive drug the specific problems of first-line treatment should be taken into account. First, high blood pressure is not a distinct disease entity and the rationale of medical

intervention is found in the knowledge that the level of blood pressure acts as a risk factor in the development of cardiovascular and renal disease. Second, an abundancy of antihypertensive drugs is already available. Therefore, this introductory chapter will first give a brief overview of the current problems which are related to these two notions. This is followed by an outline of the RAAS and of the experience with ACE-inhibitors. Subsequently, the importance of the kidney in antihypertensive treatment will be discussed and finally, the clinical pharmacology of enalapril and the purpose of this study will be described.

1.1 The treatment of high blood pressure.

The treatment of high blood pressure has shown a remarkable development in the last decades. It has been known since 1913, when Janeway reported that 81 per cent of his hypertensive patients had died within 5-10 years of follow-up, that an elevation of blood pressure leads to an increase in mortality (Janeway 1913). Up to 1949, xanthine derivatives and sedatives like phenobarbital were unsuccessfully used to alleviate the symptoms of high blood pressure. Soon after the introduction of the ganglion blockers and subsequently hydralazine and reserpine, it became clear that the prognosis of malignant hypertension and emergencies like hypertensive heart failure could be improved by lowering blood pressure (Dustan 1958). At that time the classic study of Bechgaard on the natural history of hypertension was well under way. It started in 1932 and comprised a 32-year follow-up of 1038 patients. Two important findings were; first, the higher the blood pressure, the higher the associated risk, and second, that in some otherwise asymptomatic patients the disease can progress rapidly to malignant hypertension with a poor prognosis, whereas other patients live up to old age in spite of a similar initial blood pressure level (Bechgaard 1956). Bechgaard stated that "Early differentiation is still one of the most important problems facing the clinician as it is his principal duty to protect his hypertensive patients against the development of serious vascular disease, without having to subject too many of them to troublesome treatment...". This in essention still applies today. In asymptomatic patients the level of blood pressure does not absolutely differentiate between patients with a poor and patients with a better outlook. Consequently, the problem what level to treat in these patients was formulated soon after the first antihypertensives became available. Pickering, in 1955, recommended treatment for all patients with a diastolic blood pressure persistently of 140 mmHg or over and of persistently

130 mmHg and over in patients below forty years of age and he added: "the discovery of drugs with a low nuisance value will reduce these limits." He expected on general grounds that the benefit of treatment would be proportional to the level of blood pressure and would largely remain confined to patients with a diastolic blood pressure over 100 mmHg (Pickering 1955).

An impressive amount of data has been gathered in the thirty years that followed. The association between high blood pressure and cardiovascular morbidity and mortality has been established in detail by the actuarial statistics and the Framingham study (Anderson 1958, Shurtleff 1973, Society of Actuaries 1959). The latter study also allowed stratification of risk according to age, sex, the presence of target organ damage and the presence of other risk factors. Although providing a wealth of information, these studies do not indicate what level to treat, as they showed that cardiovascular risk is proportional to blood pressure without a threshold for blood pressure-associated changes in morbidity and mortality.

By combining the data on blood pressure-associated risk with the distribution of blood pressure in the population (a bell-shaped curve skewed to the right) (Hamilton 1954) interesting conclusions can be drawn. It can be calculated that despite the relatively low risk in patients with mild hypertension, the majority of blood pressure-associated morbidity occurs in this category as they by far outnumber the patients with severely elevated blood pressure. For instance in the US 60 per cent of the cardiovascular complications that can be attributed to an elevated blood pressure occur in persons with a diastolic blood pressure below 95 mmHg (Whelton 1984). On this epidemiological evidence and in close association with the availability of more and safer drugs, treatment has been recommended for lower and lower blood pressures over the years (Peart 1983).

The benefits of antihypertensive treatment in terms of a decrease in morbidity and mortality were shown in 1967 in the first report of the Veterans Administration for men with a diastolic blood pressure of 115-129 mmHg (Veterans Administration Cooperative Study Group 1967), and for men with diastolic blood pressure of 104-114 mmHg in a second report in 1970 (Veterans Administration Cooperative Study Group 1970). The latter study, however, failed to demonstrate an improvement in mortality and morbidity in patients with a diastolic pressure of 90-104 mmHg. Between 1970 and today six large trials have focussed on the problem whether the lowering of blood pressure in mild hypertension results in an improvement of prognosis in terms of both morbidity and mortality. Two trials including a placebo group, the Australian therapeutic trial in mild hypertension and the Medical Research Council (MRC) trial, both demonstrated that active treatment

reduces the number of cerebrovascular events in mild hypertensives (Australian National Blood Pressure Study Management Committee 1980, Medical Research Council Working Party 1985). It has been calculated from these data that 800 to 1000 patient-years of treatment are required to prevent, or postpone, one major cardiovascular complication in this category of patients. The third placebo-controlled trial by the European Working Party on High blood pressure in the Elderly, has demonstrated that the reduction in morbidity and mortality by the lowering of blood pressure also applies to subjects over sixty (Amery 1985).

Of the other large trials the Hypertension Detection and Follow-up Program showed that a special hypertension treatment program reduced both cardiovascular and overall mortality as compared to the habitual care (Hypertension Detection and Follow-up Program Cooperative Group 1979). No benefit, however, was demonstrated in younger patients and in white female patients. Actual concern was raised by the results of the Oslo trial in middle-aged men. Although in this study antihypertensive treatment reduced the number of cerebrovascular events, a small increase in coronary events was found in this group as compared to the untreated patients (Helgeland 1980). This concern was aggravated by some of the findings of the Multiple Risk Factor Intervention Trial. In this study medical intervention aimed at reducing cardiovascular morbidity and mortality by the reduction of multiple major risk factors, i.e cigarette smoking, serum cholesterol and hypertension. In this study a small increase in mortality was found in the treated hypertensive men with ECG-abnormalities at entry, despite effective blood pressure reduction (Multiple Risk Factor Intervention Trial research group 1982). These results raised the question whether subtle additional effects of treatment, affecting the coronary prone metabolic profile (e.g lipid profile, potassium homeostasis, glucose tolerance) could outweigh the benefits of a lower blood pressure. Clearly, in low risk populations subtle effects of treatment become relatively more important in the risk benefit equation (Dollery 1984). Thus the question arose whether it was relevant how blood pressure was lowered (Dollery 1981). As in both the Oslo study and in the MRFIT study most patients had been treated with diuretics, a vigorous discussion developed as to the eventual adverse effects of long term metabolic changes induced by diuretic therapy (Ames 1982, Harrington 1982, Kaplan 1984).

In the MRC trial the active treatment groups were treated with a diuretic and a beta-blocker, respectively. It was hoped therefore, that the results of this trial would solve the latter controversy. The results of this trial, recently published, did not provide evidence, however, for an overall difference in

outcome between the two treated groups (Medical Research Council Working Party 1985). The International Prospective Primary Prevention Study in Hypertension compared the outcome of a beta-blocker regimen with a non-betablocker regimen, and again did not show overall differences in morbity and mortality between the two treatment regimens (IPPPSH Colloborative Group 1985). These results have contributed to the growing notion that the large trials have brought us as far as we can get. That is, they allow for an estimation of the benefit of treatment for a given blood pressure level, especially when additional risk factors are taken into account. They will not allow, however, a decision as to what type of treatment is the best (Dollery 1984). This does not mean that the type of treatment is indifferent with regard to the outcome. Most investigators and clinicians would take up the argument in favour of a thorough knowledge of individual drugs before starting life-long treatment. Furthermore, patients should be selected not only by the level of blood pressure but also by other criteria such as the presence of additional risk factors. This approach should be combined with systematic observation of the patients thus treated (Peart 1983).

At present in the Netherlands non-pharmacological measures, i.e. reduction of dietary sodium and body weight and the abandoning of cigarette smoking are recommended for persons with a diastolic blood pressure of 90-100 mmHg (Gezondheidsraad 1983). Growing evidence indicates that non-pharmalogical measures also should comprise an adequate intake of potassium, calcium, magnesium and poly-unsaturated fatty acids and fibers (Dyckner 1983, Langford 1983, McCarron 1982, MacGregor 1983b). For patients with a diastolic blood pressure of over 100 mmHg that fails to normalize with non-pharmacological measures, drug treatment is recommended. This is in striking accordance with the prediction of Pickering thirty years ago!

The recommended treatment schedule is based on the so-called stepped care regimen as published by the WHO (WHO 1983). According to this regimen either a diuretic or a beta-blocker is instituted as first drug. In general blood pressure normalizes on either monotherapy in 50-60% of the patients. The choice of the first drug depends on age and race of the patient and the presence of, for instance, ischaemic heart disease. If the blood pressure fails to normalize after a sufficient period of time, a beta-blocker or a diuretic is added. If this combination therapy fails to normalize blood pressure, several drugs can be added; these include vasodilators and centrally acting drugs.

This stepped care regimen has been challenged recently. It has been pointed out that the prominent place of the diuretics in this treatment schedule

was due to considerations some of which are no longer of primary importance today (Zanchetti 1985). One of these is the consideration that many older antihypertensive drugs required combination with a diuretic because of the development of pseudo-tolerance due to the retention of fluid (Dustan 1983). At present, however, many antihypertensives devoid of fluid retention are available. On the other hand, arguments concerning the efficacy of diuretics, the low cost and the long experience with these drugs still hold today. High withdrawal rates due to subjective side-effects in both treatment groups in the MRC trial have put into question the presumed low incidence of subjective side-effects of diuretics, and the same holds true for the beta-blockers (Medical Research Council Working Party 1981).

It has also been stated that several new classes of drugs, such as the calcium-entry blockers and the ACE-inhibitors, possess the profile that is desirable for first-line drugs. These would therefore deserve a place in the stepped care schedule. Furthermore, the concept of stepped care itself has been challenged by the suggestion that replacing an ineffective drug might be a more fruitful approach than adding other drugs when the first has not been successful (Tarazi 1985). This approach places emphasis on carefully tailoring the initial therapy to the responsiveness of the individual patient and might therefore have the advantage of increasing the proportion of patients well controlled on monotherapy. The advantages of monotherapy include a lower overall drug consumption with presumably a lower overall incidence of side effects, lower costs, and a significantly better patient compliance (Haynes 1983).

What does this all mean for a new drug, with the potential to become a commonly used first-line drug? First, in a considerable proportion of patients blood pressure fails to normalize on monotherapy, in spite of the availability of more than thirty effective antihypertensive compounds. A new drug should decrease this proportion. Second, it should be devoid of side-effects, both objective and subjective. As to the latter, there is still room for improvement as can be appreciated from the high withdrawal rates in the above-cited studies. And last but not least and probably the most important, in view of the potential wide spread use in consumers at a relatively low risk, the long term safety should be optimal.

1.2 The renin-angiotensin-aldosterone system.

1.2.1 Historical note.

In the last years of the 19th century Tigerstedt and Bergman found that an extract of rabbit renal cortex induced a pressor response when injected intravenously; they named this substance "renin" (Tigersted 1898). It was not until Goldblatt showed in his classic experiments more than thirty years later that renal ischaemia can induce hypertension, that interest was roused again in the role of renin in the pathophysiology of hypertension (Goldblatt 1934). The nature of renin as an enzyme was elucidated when it was shown that renin acts upon a plasma substrate to form a pressor peptide (Kohlstaed 1938). This peptide was named angiotonin by Page and hypertensin by Braun-Menendez (Braun-Menendez 1940, Page 1940); they compromised on angiotensin 18 years later. The presence of two different peptides, the angiotensins I and II was discovered by serendipity in the laboratory of Skeggs when the routine procedure of in vitro preparation of angiotensin was not performed correctly on a certain occasion (Skeggs 1954). The contaminant in the preparation turned out to be the angiotensin I-converting enzyme.

After the structure of angiotensin II (AII) had been elucidated and AII had been synthetized (Rittel 1957, Schwarz 1957), it was soon clear that the actual pressor substance was AII. It was Gross who, impressed by the parallel effects of sodium status on the adrenal cortex and the juxtaglomerular apparatus, first proposed that the renin-angiotensin system regulated aldosterone secretion (Gross 1958). This hypothesis was confirmed by the finding that aldosterone secretion was stimulated by a hormone of renal origin (Davis 1961) and by the finding that infusion of exogenous AII induced a rise in the secretion of aldosterone (Genest 1960, Laragh 1960).

1.2.2 Biochemistry and physiology.

Renin is a highly specific endoproteinase that is formed mainly in the juxtaglomerular cells of the renal afferent arteriole. It is released into the circulation as well as the renal lymph in response to a variety of stimuli (Davis 1976). Apart from this enzymatically active renin there are also inactive forms of renin present within the kidney as well as in the circulation. These inactive forms have a higher molecular weight than active renin. It is known that inactive renin can be proteolyzed to active renin, but the biological significance of the inactive forms of renin is as yet unclear (Sambhi 1983).

The most important stimuli for renin release are: first, the intrarenal receptors i.e, the baroreceptor in the afferent arteriole and the macula densa recep-

tor. Second, the renal sympathetic nerves, which are considered to be involved in the fine regulation of the system, and third, a variety of humoral substances including AII and vasopressin (inhibition), and the prostaglandins I_2 and E_2 (stimulation). Within the circulation renin splits off the inactive decapeptide angiotensin I (AI) from the alpha-2-globulin angiotensinogen that is formed in the liver. Subsequently, angiotensin I-converting enzyme, ACE, a dipeptidyl carboxy-peptidase, splits off a dipeptide to form the effector hormone AII. Circulating AII is broken down within minutes to the relatively inert heptapeptide AIII (Bumpus 1964, Peach 1977).

In man ACE is bound to vascular endothelium. It is particularly abundant in the pulmonary vascular bed (Lieberman 1983, Miyazaki 1984). A major part of the circulating AII is formed in transit in the lung (Oparil 1971). ACE is also present within the kidney (in particular in the proximal tubular brush border), in the brain and in a variety of other tissues (Lieberman 1983). The enzymatic activity of dipeptidyl peptidase is not confined to the cleavage of AII; it also degrades bradykinin, enkephalins and probably a variety of other peptides (Ondetti 1982).

AII is a potent vasoconstrictor, and infused intravenously it raises arterial pressure immediately (De Bono 1963, Finnerty 1962). The vasoconstrictor action of AII is potentiated by sodium loading and diminished by sodium depletion (Hollenberg 1974b, Reid 1965, Strewler 1972). The renal vascular bed is particularly sensitive to AII and responds with vasoconstriction already at subpressor doses (Aurell 1969). The reduction in blood flow is larger than the reduction in glomerular filtration rate (GFR), suggesting the renal vasoconstrictor effect occurs mainly, but not exclusively, at the level of the efferent arteriole (Aurell 1969, Bock 1958, Edwards 1983, Navar 1974, Steinhausen 1983). AII probably leads to a redistribution of renal blood flow to the inner cortical region, i.e the juxtamedullary nephrons (Aukland 1976). These renal hemodynamic effects lead to diminished excretion of sodium and water. In addition, physiological doses of AII induce sodium retention by a direct effect on proximal, and possibly also distal tubular sodium reabsorption (Johnson 1977, Harris 1984, Schuster 1984). Physiological elevations of circulating AII increase the secretion of aldosterone (Laragh 1960), again leading to increased distal tubular sodium reabsorption. In contrast with the effects of sodium depletion on the vascular effects of AII, sodium depletion enhances the effect of AII on aldosterone (Hollenberg 1974b, Oelkers 1974).

AII has several stimulating effects on the sympathetic nervous system. AII facilitates ganglionic transmission. It enhances the synthesis and release of norepinephrine from nerve endings while inhibiting re-uptake, and finally, it

stimulates the release of catecholamines from the adrenal medulla (Zimmerman 1978). The effects of AII on the central nervous system include stimulation of thirst, stimulation of vasopressin release, and stimulation of the area postrema (Severs 1973). It has been shown that within the blood-brain barrier all components of the RAAS are present, but the physiological significance of this central RAAS is as yet unclear (Paul 1983).

The cascade leading to the formation of AII, the so-called activation of the RAAS, starts with the release of renin. This occurs in the upright posture, during sodium depletion, after blood loss and in conditions characterized by a decrease of the effective circulating volume and/or a decrease in the perfusion pressure at the level of the afferent arteriole, e.g a genuine decrease in blood pressure or a renal artery stenosis (Davis 1976).

Under these circumstances activation of the RAAS contributes to the maintenance of blood pressure by the systemic vasoconstrictor effect of AII. The renal efferent vasoconstriction helps to maintain filtration pressure and hence GFR, and the increased sodium reabsorption helps to restore effective circulating volume (Hall 1980). At the other side of the spectrum, under conditions of sodium loading, the activity of the RAAS is suppressed. Modulation of RAAS activity thus allows the organism to tolerate large fluctuations in volume status without excessive changes in blood pressure and renal function.

Although the role of the RAAS in the maintenance of blood pressure in normal individuals under conditions of sodium depletion is recognized, it is still a matter of dispute whether the RAAS participates in the maintenance of normal blood pressure in the absence of sodium depletion. Investigations into the pathophysiology of hypertensive disease hallmark the discovery of the RAAS. As early as 1950 Pickering et al showed that long term infusions of renin could produce a sustained rise in blood pressure (Blacket 1950). Since then, however, a one-dimensional relationship between excess circulating renin and hypertension has only been established for the rare renin-secreting tumor (Robertson 1967) , and for the acute stage of renovascular hypertension and malignant hypertension, all states characterized by high levels of circulating renin. Essential hypertension on the other hand, can be associated with low, normal and high circulating renin levels. This has constituted substantial problems in defining the role of the RAAS in this presumably heterogenous group of patients. The availability of pharmacological tools to interfere with the RAAS, however, as will be discussed below, has contributed considerably to our knowledge in this field.

1.2.3 Interference with the RAAS as therapeutic modality.

As shown in *figure 1.1,* the RAA cascade can be specifically interfered with at different levels. Beta-blockers have proven their efficacy as antihypertensive agents, but it is doubtful whether interference with the RAAS is crucial for their mechanism of action. Of the other modes of interference, at present only ACE-inhibition provides therapeutic possibilities. Renin inhibitors, including renin antibodies, provide an important and highly specific potential, but currently are only available for intravenous administration (Haber 1983). Moreover, most agents available at present are toxic. AII-analogues are all peptides, and therefore subject to the restriction of parenteral administration. Moreover they have intrinsic agonistic properties that are not without danger in low-renin conditions.

ACE-inhibitors were the result of purposeful pharmacological design initiated by the discovery that the venom of the brazilian snake Bothrops Jararaca contained peptides that inhibited the enzyme responsible for the degradation of bradykinin and the conversion of AI. Characterization of these peptides and their binding site on the enzyme led the way to the synthesis of the peptide ACE-inhibitor teprotide and finally to a series of orally active ACE-inhibitors of whom captopril was the first (Ondetti 1977).

As a logical consequence of the assumed pathogenetic role of the RAAS in severe hypertension associated with renal or renovascular disease, captopril was first introduced in this category of patients. Indeed it proved highly effective in this type of patients and as such was a valuable addition to the therapeutic

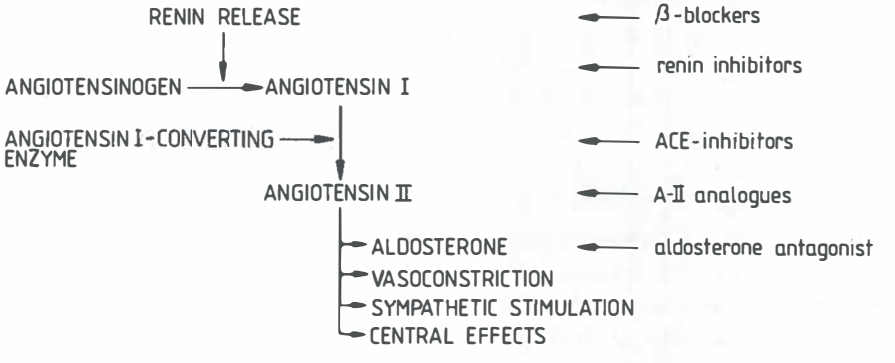


Figure 1.1: The renin-angiotensin-aldosterone cascade with the possible levels of interference.

arsenal (Atkinson 1980, Bravo 1979, Hoorntje 1981, Prins 1979b, Vidt 1982). Unexpectedly, however, captopril also lowered blood pressure, although to a lesser degree, in patients with milder forms of hypertension including many patients with low levels of circulating renin (de Bruyn 1980, 1981, Veterans Administration Cooperative Study Group 1983). Moreover, captopril lowered blood pressure in sodium-depleted anephric patients and in healthy individuals on low as well as on high sodium intake (Man in 't Veld 1980, MacGregor 1983a). It has been concluded from these findings, first, that the contribution of the RAAS in the maintenance of blood pressure is incompletely reflected by the level of circulating renin and second, that the RAAS may contribute to the maintenance of blood pressure under more conditions than previously assumed, notably including normal blood pressure in the absence of sodium depletion (MacGregor 1983b).

Even with these notions accepted, however, the mechanism of action of ACE-inhibitors is not fully clear as, moreover, a dissociation has been observed between the degree of ACE-inhibition and the effects on blood pressure (Waeber 1980). Resarch into this question has been conducted along two lines. The first line of evidence, consistent with the notion that the RAAS is not exclusively operating within the circulation, has emphasized the effects of ACE-inhibitors on the RAAS at tissue level, i.e localized in the vascular wall and in the kidney (Thurston 1977, Unger 1982). The other line of evidence has emphasized the effects of ACE-inhibitors on hormonal systems other than the RAAS, e.g. the prostaglandin- and the kallikrein-kinin system (Moore 1981, Swartz 1979, 1980).

Soon after the introduction of captopril a series of side-effects was reported that aroused wide-spread concern (Rubin 1980, Vidt 1982). These included rash, eosinophilia, taste alterations, and, more seriously, proteinuria associated with nephrotic syndrome (Hoorntje 1980, 1981, Prins 1979a) and granulocytopenia. In view of the similarity of this pattern of side-effects with those of other agents containing a sulfhydryl group like penicillamine (Bacon 1976), it was suspected that the SH-moiety of captopril molecule was involved in the genesis of these side-effects, either by a direct toxic effect or by an immunological mechanism (Kallenberg 1981). It was feared that this would seriously limit the use of captopril and future SH-containing ACE-inhibitors (Editorial 1980). This has been one of the stimuli in the development of non-SH-containing ACE-inhibitors like enalapril. Recently, however, the experiences with captopril in milder forms of hypertension have made it increasingly clear, first, that the side-effects were related to the high doses that were used in the initial years (up to 450 mg daily whereas at present the maximum dose is 150 mg daily), and second that the side effects

occur predominantly in a well-defined high-risk group, i.e patients with pre-existing renal and/or auto-immune disease (Cooper 1983, Veterans Administration Cooperative Study Group 1983). Other side-effects, like symptomatic hypotension after the first dose of captopril, and a fall in glomerular filtration rate after captopril in the affected kidney in renal artery stenosis are thought to be due to the inhibition of ACE per se (Hodsman 1983, Wenting 1984). Thus, these side-effects can be expected to occur with other ACE-inhibitors as well.

The renal effects of ACE-inhibitors have roused much interest right from their introduction. Both captopril and teprotide were reported to increase renal blood flow in a variety of species and circumstances (Hollenberg 1977, 1979, Kimbrough 1977, Zimmerman 1981), in spite of a fall in blood pressure. This effect is more pronounced after sodium depletion (Hollenberg 1981, Kimbrough 1977) and it has been shown to be more pronounced in essential hypertensives than in normal subjects (Hollenberg 1981). It has been claimed that this response is sustained on long-term treatment (Hoorntje 1981) but not all investigators confirmed this finding (Glück 1984). GFR has been shown to increase (to a lesser extent than renal blood flow), to remain unchanged or to decrease after ACE-inhibition, depending on the model studied (Zimmerman 1981). This invariably results in a fall in filtration fraction. The effects on renal hemodynamics thus appear to be due to a vasodilation, located predominantly, but perhaps not exclusively, at the level of the efferent arteriole.

An increase in sodium excretion accompanied by a decrease, no change or an increase in potassium excretion has been observed after acute ACE-inhibition (Atlas 1979, Bengis 1981, Kimbrough 1977, McCaa 1978, Zimmerman 1981). The natriuresis could be accounted for by the altered renal hemodynamics as well as the decrease in aldosterone secretion observed after ACE-inhibition (Atlas 1979). Whether this natriuresis indeed induces a negative sodium balance on continued treatment is still a matter of debate. A net sodium loss has been described to occur after institution of captopril (Atlas 1979, MacGregor 1981) as well as enalapril (de Leeuw 1983), but other investigators did not reproduce these findings (Johns 1981, Hodsman 1984, Tarazi 1980). In view of the complex actions of the RAAS and the potentiation of the renal hemodynamic effects of ACE-inhibition by sodium restriction, this disparity could be due to differences in the type of hypertension studied as well as to differences in the prevailing state of sodium balance.

1.3 Antihypertensive drugs and the kidney.

Blood pressure regulation is complex and involves the integrated action of several regulation systems. Guyton has elaborated an extensive systems-analysis of these integrated actions, which emphasized the central role of the kidney in the long term regulation of blood pressure (Guyton 1974). In brief a fall in blood pressure mediated by interference with one of these systems elicits a homeostatic response of the other systems directed at the restoration of the original level of blood pressure. When blood pressure is lowered by pharmacological intervention, different feedback mechanisms become operative in time. Nervous reflexes account for most of the short-term feed back response. These include the baroreceptor reflexes, the central nervous system ischaemic response and the chemoreceptor mechanism. Albeit potent, they only partially restore blood pressure to the original level. Moreover, they adapt to the prevailing level of blood pressure within days. Thus, mainly long-term feed back mechanisms are relevant to the maintenance treatment of hypertension.

Long term blood pressure control is determined by the relationship between arterial pressure and the renal excretion of sodium and fluid (Borst 1963, Guyton 1974). The mechanism is as follows: a decrease in arterial pressure leads to a decrease in sodium excretion (Selkurt 1951, Shipley 1951). This results in an expansion of extracellular fluid volume. This expansion of extracellular fluid volume eventually restores blood pressure to its original level. A long term reduction in arterial pressure is in this concept necessarily associated with a change in the relationship between arterial pressure and sodium excretion, irrespective of the mechanism that initiated the fall in blood pressure. If, on the other hand, such a change fails to occur, (as is for instance the case after the administration of various vasodilators), blood pressure eventually returns to its original level.

The change in the relationship between arterial pressure and sodium excretion is loosely called the re-setting of pressure natriuresis. This denomination is perhaps somewhat confusing in that it could suggest a concrete mechanism whereas de facto, it refers to a relationship. Actually, a change in this relationship can be achieved by any mechanism involved in renal sodium handling. The impact of these notions on the pharmacology of antihypertensive drugs has been pointed out by recently (Struyker-Boudier 1980). On one hand, the success of antihypertensive therapy depends on the efficacy of renal compensatory mechanisms. On the other hand, pharmacological intervention can be aimed directly at the resetting of pressure natriuresis in order to achieve a fall in blood pressure (Ackerman 1982).

The RAAS has extensive effects on renal hemodynamics and renal sodium handling. Accordingly, ACE-inhibitors exert distinct effects on renal function and renal sodium handling. It has been pointed out that the mechanism by which ACE-inhibition lowers blood pressure is not completely elucidated. In view of the abovementioned analysis it could well be that the renal effects of ACE-inhibitors contribute to their effects on blood pressure. To explore the renal effects of enalapril and to assess whether these contribute to its effects on blood pressure is one of the objectives of the present studies.

1.4 Enalapril, clinical pharmacology.

Enalapril [N-(S)-1-(ethoxycarbonyl)-3-phenylpropyl-L-alanyl-L-proline] *(figure 1.2)* is a maleate-salt (Patchett 1980) that is readily absorbed after oral ingestion (Ulm 1983). Food does not alter its absorption (Ferguson 1983). In vitro enalapril is a weak inhibitor of ACE with 50 per cent inhibition(I-50) of ACE-activity in hog plasma at 1200 nmol, whereas the I-50 of captopril is 20 nmol in the same assay (Patchett 1980). In vivo the pharmacological effects of enalapril are exerted by its active metabolite, the diacid enalaprilic acid. Enalaprilic acid is poorly absorbed in the gut, but its potency as an ACE-inhibitor by far exceeds that of enalapril with an in vitro I-50 at 1,2 nmol (Sweet 1983). The site of hydrolization of enalapril to enalaprilic acid

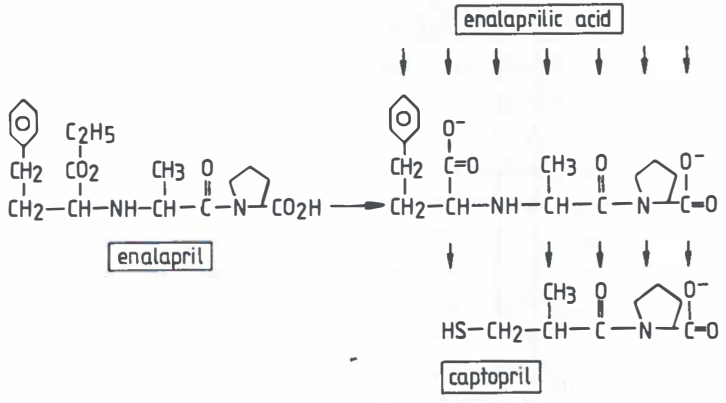


Figure 1.2: The molecular structure of enalapril, its active metabolite enalaprilic acid and captopril. Arrows indicate the binding site with the angiotensin converting enzyme.

depends on the species studied (Gross 1981). In man the site of activation is not well-defined but most probably involves the liver (Sweet 1983). Human plasma does not activate enalapril. Whether activation occurs in tissue, especially target tissue such as the vascular wall or the kidney is not known. In rats selective in vitro hydrolysis of enalapril to enalaprilic acid has been shown to occur in plasma, in the kidney and albeit less completely, in the brain but not in aortic tissue (Cohen 1983, Unger 1982). In man, the bioavailability of enalapril as enalaprilic acid is approximately 40%, but it may be different at the tissue level (Ulm 1983).

After ingestion of a 10 mg dose of enalapril the peak serum level of enalapril is reached after one hour and the peak serum level of enalaprilic acid after four hours. With daily dosing, steady state serum levels of enalapril are achieved after three to four days without evidence of accumulation thereafter (McNabb 1985, Ulm 1983). The effective half-life of enalaprilic acid after multiple dosing is approximately 11 hours. Both enalapril and enalaprilic acid are excreted by the kidney. The excretion of unchanged enalapril reaches its maximum at one to two hours after ingestion and is largely complete within four hours. A renal clearance of 300 ml/min has been calculated for enalapril, implying tubular secretion of the drug (McNabb 1985, Ulm 1983). The excretion of enalaprilic acid peaks at four to eight hours. In healthy individuals the renal clearance has been reported to be in the order of the GFR. When GFR falls below 30 ml/min, the renal excretion of both enalapril and enalaprilic acid falls steeply (Saris 1984).

The inhibition of ACE parallels the serum levels of enalaprilic acid (Biollaz 1982, Johnston 1983). In man, a 2.5 mg dose of enalapril is sufficient to lower serum ACE to below 5% of pre-treatment values. Higher doses like 5, 10 and 20 mg result in a similar decrease with a longer duration of action (de Leeuw 1983). After a 10 mg dose, both blood pressure and serum ACE-activity are still suppressed (although the decrease has passed its nadir) 24 hours after dosing. Therefore, the recommended dose of enalapril is 10 mg once daily which can be increased to 20 mg once and eventually twice daily.

After oral ingestion of enalapril the fall in blood pressure starts after one to one-and-a-half hour, with a maximum after four to eight hours (Johnston 1983). This more gradual onset of action as compared to captopril may be due to the fact that enalapril is a pro-drug. In addition animal experiments have provided evidence that slowly evolving ACE-inhibition at the tissue level also contributes to the fall in blood pressure after enalapril (Unger 1982).

PRA rises after enalapril, most probably due to the abolition of the

inhibitory action of AII on renin release (Given 1984). This rise has been shown to be sustained during long-term therapy (Gavras 1981). Plasma aldosterone falls after enalapril but returns to baseline 24 hours after dosing when both ACE-activity and blood pressure are still suppressed (Biollaz 1982). Data on the aldosterone response in long term treatment are conflicting which may in part be due to differences in the sodium status of the patients (de Leeuw 1983). AII levels are notoriously difficult to measure after ACE-inhibition. In most assays some cross-reaction occurs with AI. As AI levels are greatly increased after ACE-inhibition this seriously confounds the results (Giese 1983). Recently, a more specific assay has been developed (Nüssberger 1985). With this assay AII levels were found to be persistently reduced on long term treatment, whereas control measurements with the conventional assay failed to detect a change from pre-treatment levels.

The hemodynamic effects of enalapril are essentially similar to those of other ACE-inhibitors (apart from pharmacokinetic differences). The acute response is characterized by a fall in blood pressure due to a fall in total peripheral resistance (Fouad 1984). An increase in cardiac output has been reported due to an increase in stroke volume but other investigators found no change in cardiac output (Lund-Johansen 1984). No reflex tachycardia occurs, a finding that has also drawn attention after captopril. On long-term treatment the fall in peripheral resistance is sustained. Cardiac output has been reported to be unchanged from pre-treatment levels after 5-13 months of treatment (Lund-Johansen 1984).

It is not fully explained why reflex tachycardia does not occur after ACE-inhibition, but it might be due to an increase in vagal tone (Campbell 1985). In studies on long term treatment of essential hypertension the blood pressure and heart rate responses to upright posture and dynamic exercise were intact (Lund-Johansen 1984). The autonomic reflexes after Valsalva manoeuvre, exercise and cold pressor test have been found to be unimpaired after enalapril in salt replete healthy individuals (Millar 1982). It has been shown in mildly salt-depleted healthy individuals, however, that the baroreceptor response to tilting is impaired after enalapril (Ibsen 1983). The observation of hypotension and bradycardia afer enalapril in patients with congestive heart failure suggests, that an interaction of ACE-inhibition with the autonamic reflexes can become clinically relevant in this condition (Cleland 1985).

As already mentioned, in acute experiments as well as on long term treatment the inhibition of plasma ACE-activity parallels the serum levels of enalapril (Biollaz 1982, Johnston 1983). It has been reported by these authors that the blood pressure response is correlated to the decrease in plasma ACE-

activity. This has been taken to imply that the blood pressure response to enalapril can be sufficiently accounted for by the decrease in circulating AII-levels. This assumption, however, is challenged by two types of observations. First, the dose-response for the antihypertensive effect of enalapril as well as other ACE-inhibitors is flat, unlike the dose-response for serum levels and for ACE-inhibition. Thus, after a 40 mg dose of enalapril serum levels of enalaprilic acid are higher than after a 10 mg dose, and the degree of ACE-inhibition is more pronounced. The blood pressure response, however, is not increased but prolonged. The second objection stems from the observation that in several animal models the blood pressure response does not run parallel with the inhibition of the pressor response to AI (Sweet 1981). In the spontaneously hypertensive rat, enalapril elicits a bi-phasic blood pressure response with an initial peak corresponding to the inhibition of the AI pressor response and a second peak at five to six hours when the inhibition of the AI pressor reponse is minimal. Strikingly, in this model, enalapril, in doses that were equipotent with captopril with respect to the inhibition of the AI pressor response, was more potent in the lowering of blood pressure. It has been suggested, therefore, that enalapril (a more polar molecule than captopril) has better access to tissue sites of action, accounting for the secondary decrease in blood pressure (Unger 1982). In yet another model, the 2-kidney Grollman renal hypertensive rat, a tenfold higher dose of enalapril was required to lower blood pressure than to block the pressor response to AI (Sweet 1981). These findings strongly suggest that a decrease in circulating AII is not the only mechanism of action of ACE-inhibitors, but the implications of these findings for the mechanism of action of enalapril in human essential hypertension are as yet unclear.

1.5 Scope of the study.

This study deals with the effects of enalapril and its active metabolite, enalaprilic acid, in the treatment of essential hypertension with special emphasis on the renal effects. The effects of enalapril on renal hemodynamics and sodium excretion were studied to assess whether they contribute to the effects of enalapril on blood pressure.

As to this question, we studied the effects of enalapril treatment on renal hemodynamics and sodium excretion in essential hypertension in relation to the antihypertensive effects. In addition, when studying the effects of enalapril on renal hemodynamics, sodium excretion and blood pressure, we tried to establish whether these effects were specific for interference with the RAAS. This question of specificity was approached along several lines of investigation. We investigated whether the effects of enalapril were influenced by the prevailing state of activation of the RAAS; we investigated whether exogenous AII could abolish the effects of enalaprilic acid, and we investigated whether the renal effects of long term treatment with enalapril were different from those of a conventional regimen with a similar effect on blood pressure.

The net effect of any antihypertensive therapy is the resultant of the intrinsic properties of the pharmacological agent and the counterregulatory response of the body, both of which are not constant over time. The study is, therefore, concerned with three shifts of time. First, the acute effects as studied after a single injection of the active metabolite enalaprilic acid. Second, the short term effects of institution of enalapril and finally, the long term effects of maintenance treatment.

The study protocols and the methods are described in chapter two. The effects of enalapril and enalaprilic acid on blood pressure in the different studies are described in chapter three, the effects on renal hemodynamics in chapter four, and the effects on sodium excretion in chapter five. Finally, chapter six discusses the evidence linking the renal effects to the effects on blood pressure and the evidence concerning the specificity for interference with the RAAS.

CHAPTER 2 PATIENTS, PROTOCOLS AND METHODS.

2.1. Patients.

The patients enrolled in our studies were between 30 and 70 years of age. They all had essential hypertension; secondary hypertension was excluded by history, physical examination, rapid sequence urography and, if appropriate by additional investigations. Renal function had to be normal or only slightly impaired, defined as a creatinine clearance greater than 70 ml/min. Urinalysis had to be normal. The patients had to be free of signs and symptoms of heart failure. Liver function, as assessed by blood chemistry, had to be normal. Patients with evidence of auto-immune disease were not included, either were patients with diabetes mellitus. Concomitant medication that could affect blood pressure was not allowed. Pregnant or nursing women and women in their fertile years who did not follow a medically accepted form of pregnancy prevention were excluded, as were women receiving hormonal contraceptives.

Informed consent, as required in the Declaration of Helsinki, was obtained in all patients. The study was approved by the Ethical Committee of the University Hospital.

2.2. Protocols.

Two categories of protocols were carried out. In the first category the effect of intravenously injected enalaprilic acid was studied. In the second category both short-term and long-term effects of orally administered enalapril were studied. In both categories the studies focussed on blood pressure, renal hemodynamics and sodium excretion.

2.2.1. ENALAPRILIC ACID PROTOCOLS.

2.2.1.1. Effects on blood pressure.

Three series of experiments were carried out. The first was aimed at dose-finding. The second series, the furosemide study, aimed at investigating the blood pressure response to enalaprilic acid after preceding renin stimulation by injection of furosemide. The third series focussed on the response to enalaprilic acid after a longer period of renin stimulation by dietary sodium restriction.

Throughout the studies the patients were hospitalized. Two weeks earlier they had been instituted on a diet containing 100 mmol of sodium in the dose-finding study and the furosemide study, and on a diet containing either 50 or 200 mmol sodium in the sodium restriction study. Potassium intake was standardized at 100 mmol daily and fluid intake was 2500 ml in all studies. Compliance was assessed by 24-hour urine collections troughout the studies.

Concomitant anti-hypertensive medication was withdrawn at least two weeks prior to the study in all patients with the exception of one patient with an inacceptable high blood pressure. This 46-year old man, who participated in the dose-finding study, received diuretic treatment throughout the study.

Experimental protocol *(figure 2.1).* This protocol was used in all studies on the effects of enalaprilic acid, with modifications as required by the particular protocol (vide infra). From 10 p.m. the evening before until 10 p.m. on the experimental day the patients were recumbent. Intake of food and fluids was distributed over the day in small isocaloric portions, that were equal with respect to sodium, potassium and fluid content. At 7.30 a.m. a catheter was

inserted into a forearm vein. This catheter was used for the infusion of the pharmaceuticals. In order to establish constant diuresis for the concomitant renal function studies 150 ml glucose 5 per cent was administered per hour. Blood pressure was measured hourly (Dinamap®) from 8 a.m. until 10 p.m. From 15 minutes prior to the drug injection (at noon) to one hour after injection measurements were made every 2 minutes. Immediately prior to every drug injection and one and three hours after injection blood samples were drawn for the determination of PRA and PAC. From 8 a.m. to 10 p.m. the patients voided at one-and two-hour intervals for the determination of the urinary excretion of sodium, potassium and phosphate. When renal function studies were performed, the sampling of blood and urine for the measurement of ERPF and GFR ran from 10 a.m. to either 5 p.m. or 10 p.m.

2.2.1.1.1. Dose finding.

This study was carried out in nine patients, four male and five female, age 35 to 57 years. The study consisted of four experiments per patient, always separated by at least two days wash-out. Its aim was to find the dose appropriate for effective and safe blood pressure reduction, within a pre-set dose range of

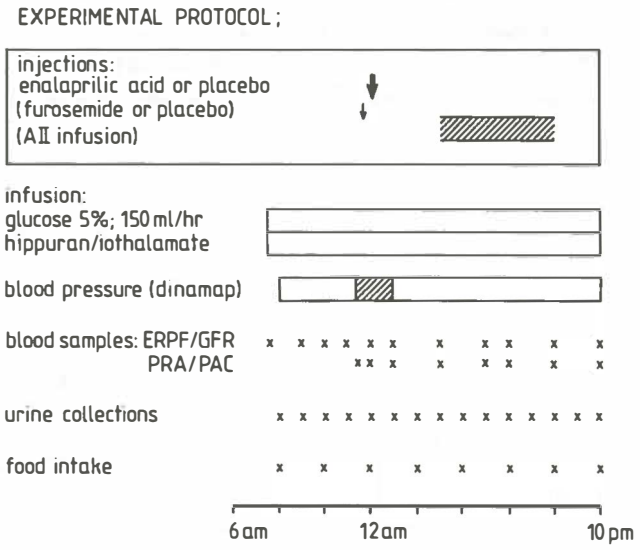


Figure 2.1: Global experimental protocol. This global protocol was used in all studies on the effects of enalaprilic acid, with modifications according to the particular study (see text). Furosemide and AII were administered only in the studies concerned with their particular effects. The sampling for the renal function studies ended at either 5 p.m. or 10 p.m. The sampling for the determination of urinary electrolyte excretion always ran from 8 a.m. op to 10 p.m.

1.25 to 80 mg. To this purpose a titration criterion was chosen, defined as a decrease in mean arterial pressure of more than 15 per cent and a decrease in diastolic blood pressure of more than 10 per cent within 10 minutes after administration. If the fall in blood pressure did not exceed this criterion after 10 minutes, a similar second dose was administered at that time and on the following experiment (two to three days later) the dose was doubled *(figure 2.2, continuous lines)*. If the criterion was exceeded, the patient received a lower dose on the next experiment *(figure 2.2, broken lines)*.
This titration should, by virtue of its up and down design, lead to a clustering of doses around the most suitable dose. The starting dose in the first three patients was set at 2.5 mg, which could be repeated after 10 minutes whenever the titration criterion was not reached (i.e. 2 x 2.5 mg). For the next three patients and the last three patients the starting dose was determined by the response on the starting dose of the preceding patients. As in none of the first three patients the criterion was reached on the starting dose, the next three patients started with 5 mg which could be repeated after ten minutes. As in neither of these patients the criterion was reached on the first dose, the last three patients started with 10 mg, which could be repeated after 10 minutes. The protocol did not allow the administration of doses below 1.25 or above 80 mg although application of the titration rules could lead to these doses. In such a case the dose on the previous experimental day, i.e. 1.25 or 2 x 40 mg, respectively, should be repeated. The results of this section are given in 3.1.1.

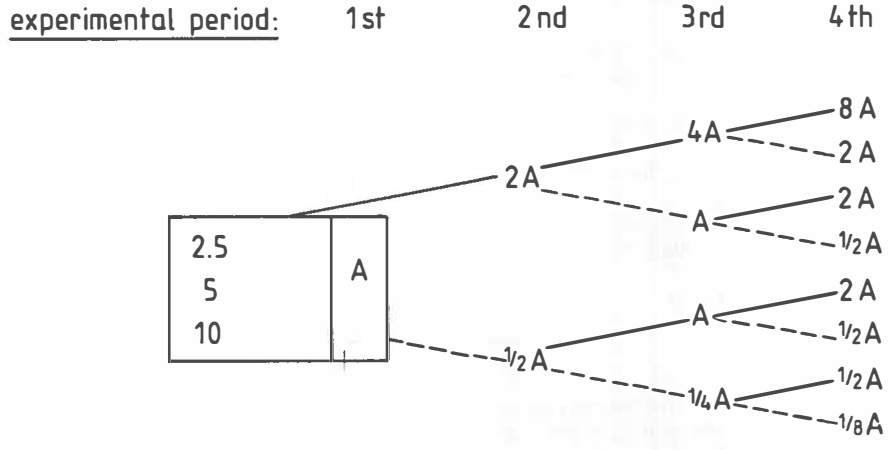


Figure 2.2: Titration schedule of the dose-finding study. It shows the possible doses each patient could receive. Patients start with (2x)2.5, (2x)5, or (2x) 10 mg. After each dose the next dose is titrated either upward (continuous lines) or downward (broken lines).

2.2.1.1.2. Pre-treatment with furosemide

This study was carried out in seven patients, four male and three female, age 30 to 70 years. Per patient this study comprised five experiments, always separated by at least two days wash-out. The experiments had a fixed sequence, shown in Table 2.I. On the successive experimental days the patients received: day A: placebo; day B: enalaprilic acid 10 mg; day C: furosemide 40 mg i.v. followed after 15 minutes by enalaprilic acid 10 mg i.v; day D: enalaprilic acid 10 mg and day E: furosemide 40 mg i.v. followed after 15 minutes by an injection of placebo. Blood samples were drawn for determination of PRA immediately prior to each injection. Urine collections were made for the urinary excretion of sodium. On wash-out days after a study including furosemide, patients received a saline infusion that was balanced to compensate for the sodium loss the day before. The results of this section are given in 3.1.2.

Table 2.I Sequence of experiments in the furosemide pre-treatment study.

Experiment		Injections. 11.45h	12.00h
1st	A		placebo
2nd	B		enalaprilic acid
3rd	C	furosemide	enalaprilic acid
4th	D		enalaprilic acid
5th	E	furosemide	placebo.

2.2.1.1.3. Sodium restriction.

This study was carried out in five patients, two male and three female, age 38 to 54 years. In this study the patients received two times a dose of 10 mg enalaprilic acid; one when they were in balance after at least a week on a 50 mmol sodium diet and one when they were in balance on a 200 mmol sodium diet, in a randomized sequence. The results of this section are given in 3.1.3.

2.2.1.2. Effects on renal function and sodium excretion.

Parallel to the studies on the effects of enalaprilic acid on blood pressure, we evaluated its renal effects. On the experimental days described in 2.2.1.1., renal hemodynamics and electrolyte excretion were assessed before and after administration of enalaprilic acid. Both the acute effects of enalaprilic acid and the effects of an infusion of A II following the injection of enalaprilic acid were studied. The results of this section are given in 4.1.1 and 5.1.1.

2.2.1.2.1. Acute effects.

In fourteen patients, seven male and seven female, age 30 to 57 years, the effects of enalaprilic acid on renal hemodynamics and sodium excretion were assessed; in 3 patients after a dose of 5 mg and in 11 patients after a dose of 10 mg. The effects on ERPF and GFR were monitored up to 5 p.m. in nine patients and up to 10 p.m. in five patients.

The effects of placebo on renal function and sodium excretion were studied in 6 of these patients, in order to account for non-drug-specific effects. In these studies ERPF and GFR were monitored up to 10 p.m.

2.2.1.2.2. Effects of AII on the renal response.

In five patients, who participated in the abovementioned study (2.2.1.2.1.) a second study was carried out after a wash-out period of at least three days. They received 10 mg enalaprilic acid again, followed by a graded infusion of AII (Hypertensin®). This infusion was given to assess the role of decreased formation of AII in the effects of enalaprilic acid on renal hemodynamics and electrolyte excretion. To this purpose an infusion of AII was started at the moment the responses to enalaprilic acid of both renal hemodynamics and sodium excretion leveled off at their maximum (Tmax). Tmax was established for each individual from the previous study day with enalaprilic acid. It was three hours in one patient and five hours in the four remaining patients. The AII infusion started at a rate of 0.1 ng/kg/min. Blood pressure was measured every two minutes. The infusion rate of AII was stepwise increased at five-minute intervals until blood pressure was similar to baseline level. Then the infusion rate was kept constant for two hours. After two hours the infusion was stopped and the recovery was studied for another two to four hours.

Blood samples for determination of PRA and PAC were drawn before the enalaprilic acid injection, at the start of the AII titration, at the end of the AII infusion, and two hours post AII. On these study days we also measured lithium clearance as an index of proximal tubular sodium handling (Thomsen 1984). To this purpose the patients had received lithiumcarbonate 300 mg orally at 11 p.m. the evening before.

2.2.2. ENALAPRIL PROTOCOLS.

In this part we studied the effects of oral maintenance treatment with enalapril. Two types of studies were carried out. In the first type the effects of long term treatment with enalapril on blood pressure and renal hemodynamics were compared with those of conventional treatment. In the second the effects of dietary sodium restriction on the responses of blood pressure and renal hemodynamics to short term treatment with enalapril were assessed.

2.2.2.1. Long term treatment.

2.2.2.1.1 Effects on blood pressure; efficacy and safety.

For this out-patient study the patients visited our out-patient clinic at two- and four-week intervals for 26 weeks. The protocols are depicted in *figure 2.3.* Previous anti-hypertensive medication was withdrawn for at least two weeks, or so much longer as was required to obtain stable blood pressure on placebo therapy. The patients were advised to adhere to a diet containing 100 mmol of sodium and 100 mmol of potassium daily. Compliance was assessed by 24-hr urine collections obtained the day before every visit. After stabilization on placebo the patients were randomly and double-blindly assigned to either an enalapril-regimen or a propranolol-regimen. Twenty-four patients with diastolic blood pressure (DBP) 96-115 mmHg on placebo were treated according to the "mild-to-moderate hypertension titration schedule".

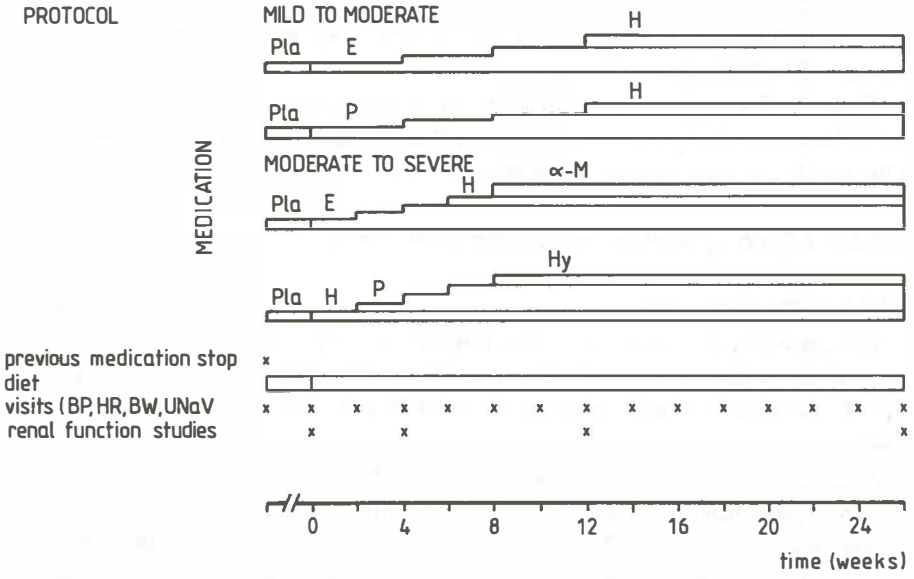


Figure 2.3: Global protocol of the outpatient studies with enalapril according to the mild to moderate titration schedule (upper panel) and according to the moderate to severe titration schedule (lower panel). The medication could be titrated upward (see text) at four- and two-week intervals respectively; this was done if diastolic blood pressure exceeded 90 mmHg.
Pla=placebo; E=enalapril; P=propranolol; Hy=hydralazine; α-M=alpha-methyldopa.

This implied that the patients started with either enalapril 5 mg bid or propranolol 40 mg bid, which could be increased at 4-week intervals to 10 and 20 mg bid or 80 and 120 mg bid, respectively. Thereafter hydrochlorothiazide could be added to either medication.

Nineteen patients with DBP 106-130 mmHg were treated according to the "moderate-to-severe hypertension titration schedule". This implied that they started with either enalapril 5 mg bid or hydrochlorothiazide 50 mg. Medication could be increased at two-weeks intervals to 10 and 20 mg enalapril bid with the subsequent addition of hydrochlorothiazide and alpha-methyldopa if needed. In the propranolol group, propranolol 40, 80 and 120 mg bid could be added subsequently to the hydrochlorothiazide, followed by addition of hydralazine. In both the mild-to-moderate and the moderate-to-severe schedule, the titration criterion was a DBP of 90 mmHg or less. The results of this section are given in 3.2.1.

2.2.2.1.2. Renal function.

In the patients described under 2.2.2.1.1, renal function studies were done prior to institution of therapy and after 4, 12 (n=41) and 26 weeks (n=35) of therapy. The 35 patients studied after 26 weeks included two patients not studied at 12 weeks. In a subset of the patients on enalapril or propranolol additional renal function studies were done before the addition of hydrochlorothiazide. The results of this section are given in 4.2.1.

2.2.2.2. Effects of sodium restriction; short term.

2.2.2.2.1. Blood pressure.

Ten patients, five male and five female, age 38 to 52 years were studied according to the protocol depicted in *figure 2.4.* Renal function had to be strictly normal in these patients, i.e. a GFR >90 ml/min/1.73 m^2 (ter Wee, 1986).

After the previous medication had been withdrawn for at least 4 weeks, the patients were hospitalized. They were instituted on a rigidly standardized diet with either a low or a liberal sodium-content (50 mmol and 200 mmol of sodium per day, respectively) with standardized potassium- and fluid intake (100 mmol and 2500 ml per day, respectively). After a run-in period of at least a week, when 24-hour sodium excretion, body weight and blood pressure had stabilized, treatment with enalapril 10 mg bid was instituted for eight days. During the run-in as well as during treatment 24-hour urine was collected continuously for determination of the excretion of sodium, potassium, creatinine and phosphate. Blood pressure was measured daily (Dinamap®). Blood

samples for the determination of PRA and PAC were drawn daily, just before the morning dose of enalapril was taken. Body weight was measured daily, immediately after breakfast. After eight days the medication was withdrawn. The observations were extended up to seven days after withdrawal in eight out of ten patients. Then the patients were crossed over to the other diet and the whole study was repeated. At least three weeks separated both treatment periods. The results of this section are given in 3.2.2 (blood pressure) and 5.2.1 (sodium excretion).

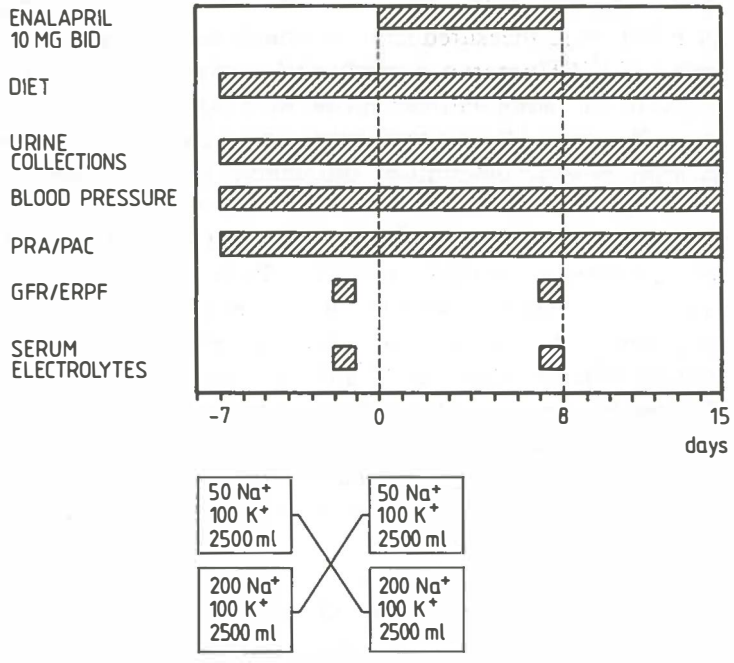


Figure 2.4: Protocol of the cross-over study on the effects of sodium restriction on the effects of enalapril on blood pressure, renal hemodynamics and sodium excretion.

2.2.2.2.2. Renal function.

In nine out of these ten patients ERPF and GFR were measured during the run-in period and after eight days of treatment on either diet. The results of this section are given in 4.2.2

2.3. Methods.

In the out-patient studies blood pressure was measured with a standard mercury sphygmomanometer. Measurements were performed in triplo after 10 minutes of supine rest in the supine and in the standing position. The mean of three readings, differing not more than 10 mmHg, was recorded. In the in-patient studies blood pressure was recorded with a semi-automatic non-invasive device (Dinamap®) (Silas 1980). For every determination blood pressure was measured at one-minute intervals for 20 minutes with the patient in the supine position. The mean of the last 10 readings as recorded. GFR and ERPF were measured simultaneously as the clearances of ^{125}I-iothalamate and ^{131}I-Hippuran, respectively (Donker 1977). After a priming dose was given, the radiopharmaceuticals were infused at a constant rate (Braun Unita II pump). After an equilibration period of 1.5 hour, subsequent hourly clearances were determined throughout the observation period. These clearances were calculated from the urinary tracer excretions and the serum tracer values by using both the amount of infused tracer and the amount of excreted tracer in the formulas IxV/P and UxV/P. During the procedure a diuresis of at least 100 ml/h was maintained by intravenous administration of glucose 5 percent. The coefficients of variation of the day to day determinations were calculated as 2.2 and 5 per cent, respectively (Donker 1977). Filtration fraction was calculated as the ratio GFR/ERPF. The values of ERPF and GFR were corrected for standard body surface area. PRA and PAC were determined by radioimmunoassay (Freedlander 1974, Pratt 1978). Serum and urinary electrolytes were measured by standard auto-analyzer technique.

Data are generally presented as means ± SEM. Data on small subsets of patients are given as median and range. Mean arterial pressure is calculated as diastolic blood pressure plus 1/3 x pulse pressure. In the experiments concerning the effects of enalaprilic acid, baseline blood pressure was calculated as the mean of the values between 10 and 12 a.m., and doses are given as the total dose administered per experiment. Statistical evaluation, when appropriate, was carried out by means of the Wilcoxon two-sample test and the Wilcoxon test for paired data. Results were considered statistically significant at the 5 per cent level.

CHAPTER 3. BLOOD PRESSURE.

Introduction.

The effects of enalaprilic acid and enalapril on blood pressure are the subject of this chapter. The effects of intravenously injected enalaprilic acid on blood pressure were studied with two different objectives. First, to establish whether this agent could be a useful drug for rapid blood pressure reduction in patients with moderate to severe hypertension. Second, to investigate whether manipulation of the state of activation of the RAAS affects the blood pressure response to acute ACE-inhibition. To explore the latter question we studied the effects of acute pharmacological stimulation of the RAAS (i.e. injection of furosemide) as well as physiological stimulation (i.e dietary sodium restriction) on the blood pressure response to enalaprilic acid.

At the time of initiation of these studies enalapril had already been shown to lower blood pressure effectively (Gavras 1981). Therefore we tried to establish its efficacy as a first step in antihypertensive treatment in comparison to conventional treatment. Furthermore, we investigated whether preceding physiological stimulation of the RAAS by a moderate restriction of dietary sodium augments its efficacy.

Finally, it has been pointed out already that one of the main objectives of these studies was to investigate whether the renal effects of ACE-inhibition contribute to their antihypertensive effects. This question will be dealt with in chapter 6, after the effects on renal hemodynamics and on sodium excretion have been given in detail in the chapters 4 and 5.

3.1 Effects of enalaprilic acid.

3.1.1 Dose finding

This study aimed at defining the optimal dose for rapid blood pressure reduction. To this purpose nine patients received four doses enalaprilic acid according to the up-and-down titration schedule described in 2.2.1.1 (figure 2.2) The dose range studied was 1.25 to 80 mg.

Initial values of blood pressure, PRA and PAC on the subsequent experimental days are given in table 3.I. Initial blood pressure did not change significantly in the course of the study. Initial PRA increased significantly and initial PAC also showed a rise that reached statistical significance at the third experiment.

Table 3.I Initial values. (n=9, mean ±SEM)

Initial values in the subsequent experimental periods of mean arterial pressure, PRA and PAC.

	MAP (mmHg)	PRA (nmolAI/l/h)	PAC (nmol/l)
experiment			
1st	125(115-135)	0.2 (0.2-1.9)	0.30 (0.22-0.70)
2nd	122(116-137)	0.7* (0.2-2.8)	0.31 (0.22-0.70)
3rd	121(116-131)	1.1* (0.2-4.8)	0.49* (0.25-1.01)
4th	120(112-130)	1.0* (0.2-5.1)	0.44 (0.27-0.79)

*: $p<0.05$, paired test versus value at the first experimental period.

The nine patients received four times a dose of enalaprilic acid. After five out of the 36 injections, the titration criterion (i.e. a decrease in mean arterial pressure of at least 15 per cent and a fall in diastolic blood pressure of at least ten per cent) was reached within 10 minutes. This response occurred after administration of 20 mg in two patients of the second group of three, after 10 mg in one patient of the third group of three and after 5 mg (two times) in this same patient. The consequent down titration in these patients resulted in the dose distribution shown in *figure 3.1.* It shows, that the titration criterion was never reached with doses below 5 mg and that increasing the dose above 20 mg did not result in a greater number of responders.

When the titration criterion was not reached within 10 minutes, a second bolus injection, equal to the first one, was administered. Consequently, the total dose was doubled. The individual reactions to the total dose administered are depicted for all doses in *figures 3.2 and 3.3. Figure 3.2.* shows that after enalaprilic acid blood pressure decreased within 10 minutes on all doses used.

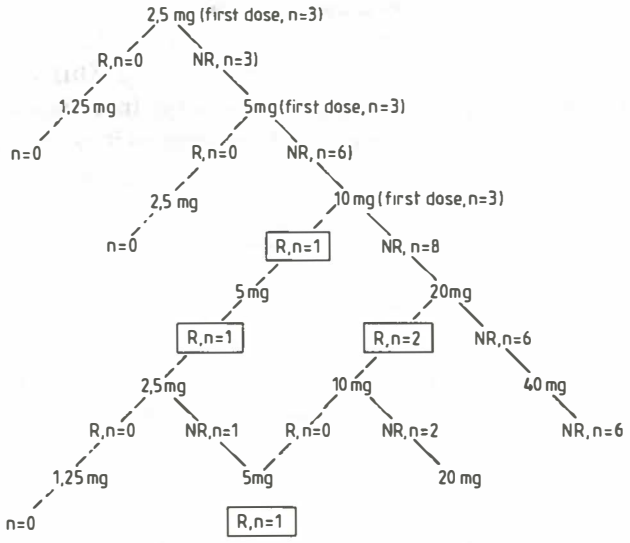


Figure 3.1: *Distribution of doses enalaprilic acid administered according to the titration criteria. If the fall in blood pressure after a given dose exceeded the titration criterion (a decrease in MAP greater than fifteen per cent and a fall in diastolic blood pressure greater than ten per cent) the reaction was called response (R) and the next does was halved. If not, the reaction was a non-response (NR), and the next dose was doubled.*

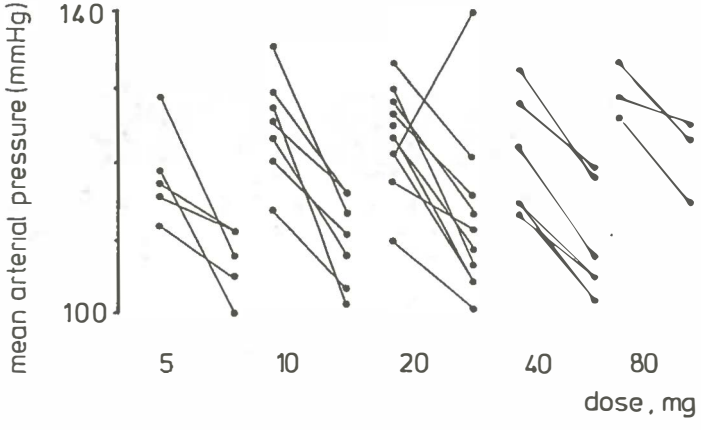


Figure 3.2: *Individual reactions of MAP to the different doses of enalaprilic acid 10 minutes after injection. In one patient a pressor response was found on one occasion. This coincided with an accident in the ward; from one hour after injection onwards a depressor response was found again in this patient.*

The magnitude of this immediate response did not increase on the higher doses, and either did the magnitude of the response after one and four hours respectively and the maximum response *(figure 3.3)*. This was also true for individual patients. Thus, within the dose range investigated the dose response curve is flat. From the range of the responses it can be read that there was a considerable variability of the response over the whole dose range. All individuals had a clear-cut fall in blood pressure on at least one occasion, so we were not able to attribute the minor responses to individual characteristics.

The duration of the response, defined as the number of hours that MAP was more than 10 per cent below baseline varied considerably. No dose-related increase in the duration of the response was observed in the dose range of 5 to 40 mg. However, in all three patients who received 80 mg, MAP was still clearly below baseline 24 hours after injection. The time elapsed between injection and the maximum response also varied considerably in the dose range of 5 to 40 mg. The maximum response could occur within 10 minutes as well as three to ten hours after injection. Only after the 80 mg dose, all maximum responses occurred late(i.e 10 hours after injection). No side-effects were observed after any injection. Nothwithstanding marked decreases in blood pressure in some patients, no symptomatic hypotension occurred. Orthostatic hypotension, however, could not be detected in our study as the patients were supine throughout the study.

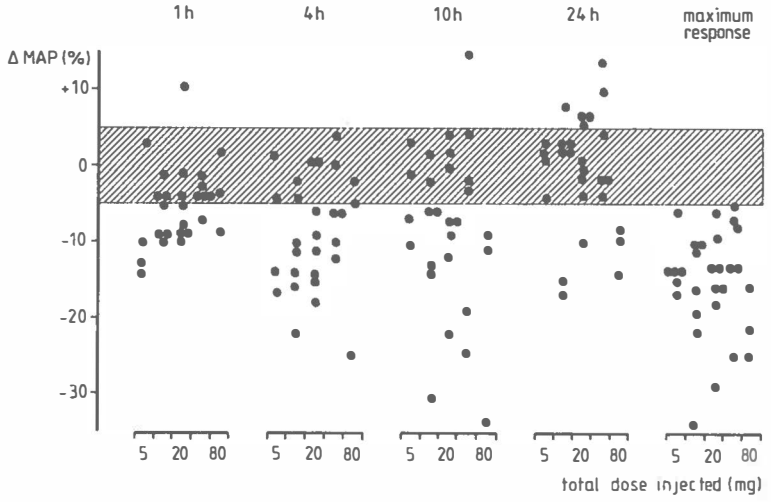


Figure 3.3: Individual reactions to the different doses enalaprilic acid, depicted as the percentage decrease in MAP at one, four, ten and twenty-four hours after injection, and the maximum response.

3.1.2 Pretreatment with furosemide.

In this study we investigated whether it is possible to augment the blood pressure response to enalaprilic acid by preceding stimulation of the RAAS with furosemide i.v. Therefore the blood pressure response to enalaprilic acid 10 mg was compared with the response to enalaprilic acid 10 mg preceded by furosemide 40 mg i.v. in seven patients. To account for non-drug-specific effects we also included a placebo experiment. Five experiments were carried out in each patient, described in detail in 2.2.1.1.2.

Initial values of blood pressure and hormonal parameters on the subsequent experimental days are given in table 3.II. Initial blood pressure tended to fall in the course of the study, but the decrease reached no statistical significance. Neither initial PRA nor PAC showed consistent changes in the course of the study. Table 3.II also gives the effects of the furosemide injection preceding enalaprilic acid. PRA increased in six out of seven patients; it decreased from 0.4 to 0.3 nmolAI/l/h in one patient. In all patients diuresis and natriuresis increased sharply within 10 minutes after injection of furosemide.

Table 3.II Initial values in the furosemide study.
(n=7, median, range)

Initial values of mean arterial pressure, PRA and PAC on subsequent experimental days, and the changes in these parameters induced by pretreatment with furosemide.

Exp	time	test drug	MAP (mmHg)	PRA (nmolAI/l/h)	PAC (nmol/l)
1	12.00	placebo	128(112-129)	1.2(0.3-3.0)	0.51(0.37-1.08)
2	12.00	enalaprilic acid	129(111-136)	0.8(0.2-3.7)	0.77(0.37-1.45)
3	11.45	furosemide	126(105-139)	1.8(0.4-4.4)	0.74(0.39-1.36)
	12.00	enalaprilic acid	123(114-137)	3.0(0.3-5.3)*	0.77(0.37-1.29)
4	12.00	enalaprilic acid	120(111-130)	1.2(0.3-4.0)	0.60(0.26-1.04)
5	11.45	furosemide	122(103-132)	1.2(0.3-3.8)	0.54(0.28-1.00)
	12.00	placebo	122(107-132)	3.4(0.6-4.6)**	0.43(0.31-0.95)

*: $p<0.05$, **: $p=0.02$, paired test versus value at 11.45h.

The blood pressure responses on the different experimental days are given in *figure 3.4* (upper panel). After placebo, a small decrease in blood pressure was observed only during the first hour ($p<0.05$). After injection of furosemide

plus placebo, a small increase in blood pressure was observed during the first hour ($p<0.05$). Both after the first and after the second single enalaprilic acid injection a prompt decrease in blood pressure was found ($p=0.02$ and $p<0.05$ resp.) with a gradual further decrease during the next three hours. On both occasions blood pressure partially returned to baseline values in the evening (after seven to ten nours). The injection of enalaprilic acid preceded by furosemide led to a fall in blood pressure that was comparable to and statistically not different from either of the single enalaprilic acid injections. In *figure 3.5* (left panel), the individual responses after enalaprilic acid alone (one and four hours after injection and the maximum response) are compared with the responses to furosemide followed by enalaprilic acid. The responses to enalaprilic acid alone were similar to the response to furosemide plus enalaprilic acid both in patients in whom the response to enalaprilic acid alone was relatively small and in patients in whom it was more pronounced. In particular the maximum responses were quite similar

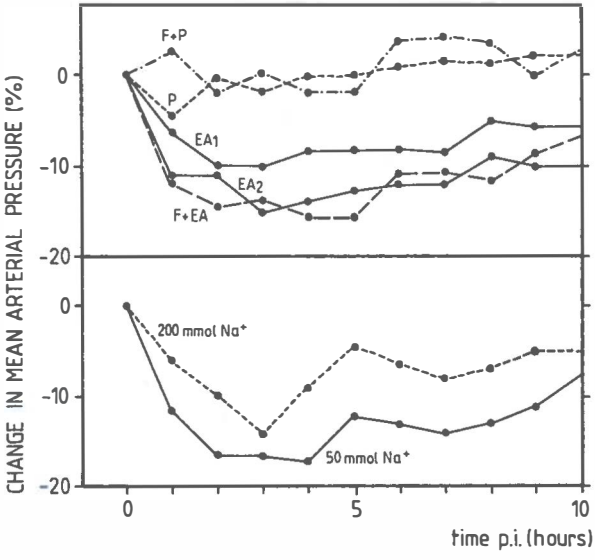


Figure 3.4: *Percentage decrease in MAP after the different injections in the furosemide study (upper panel) and in the sodium restriction study (lower panel) up to 10 hours after injection. Median values are given.*
F=furosemide, P=placebo, EA=enalaprilic acid.

Heart rate showed no significant changes after either of the injections of enalaprilic acid alone, nor after furosemide alone or after placebo. After the combination of furosemide and enalaprilic acid an increase in heart rate of 6% (-1 to +27%, $p<0.05$) up to three hours post injection was noted. No side effects, in particular no symptomatic hypotension, occurred after any of the injections.

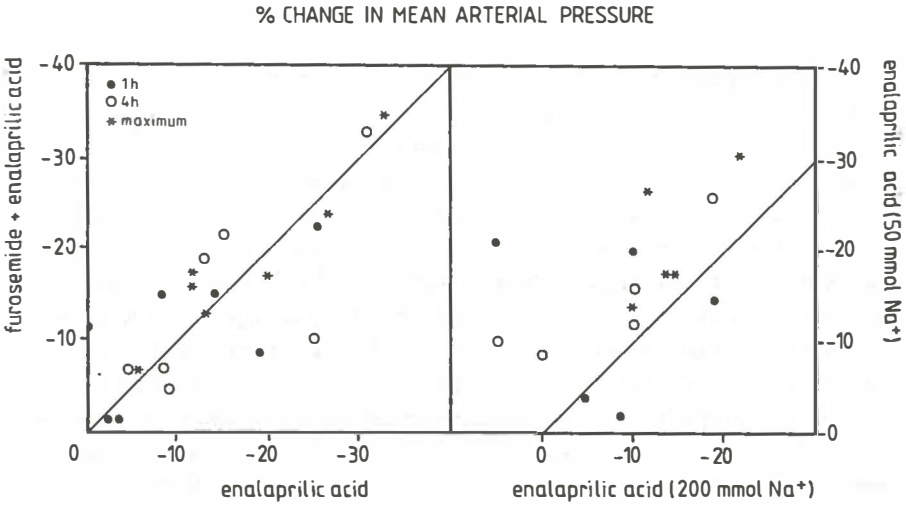


Figure 3.5: Percentage decrease in MAP after enalaprilic acid alone (mean of two injections) as compared to the combination of furosemide and enalaprilic acid (left panel) and the percentage change in MAP after enalaprilic acid on liberal sodium as compared to low sodium (right panel). In both panels the line of identity is drawn. Data are given for the response after one hour (closed circles), after four hours (open circles) and for the maximum response (asterisks)

3.1.3 Sodium restriction.

The aim of this study was to investigate whether a moderate restriction of dietary sodium augments the blood pressure response to enalaprilic acid. Five patients were studied. All received two times a dose of 10 mg enalaprilic acid; one when they were in balance on a 50 mmol sodium diet, and one when they were in balance on a 200 mmol sodium diet in a randomized sequence. Initial mean arterial pressure was similar on both diets: 103 (94-116) and 101 (88-121) mmHg on low and liberal sodium, respectively. Initial PRA was higher on low sodium than on liberal sodium in four out of five patients and similar in one patient (3.1(0.5-6.8) versus 1.0(0.4-1.7) nmolAI/l/h, respectively). The blood pressure responses are given in *figure 3.4* (lower panel). A comparison of the individual responses is given in *figure 3.5* (right panel).

Both the response after four hours and the maximum response, but not the response after one hour, were slightly more pronounced on low sodium in all patients.

Correlation of initial PRA with blood pressure response.

Data of all patients studied on a 100 mmol sodium intake (n=15) were analyzed for correlates of blood pressure response. Per patient only the data of the first experiment were included and the patient who participated in both studies was only included once. The percentage fall in mean arterial pressure was significantly correlated with the log of initial PRA, at all times up to seven hours post injection. This correlation was strongest at 10 minutes after injection ($r=0.70$; $p<0.01$). Thus the patients with the highest initial PRA had the greatest fall in blood pressure. The maximum response, however, was not significantly correlated with the log of initial PRA. When the data of the furosemide group (n=7) were analyzed separately we again found a highly significant correlation between the log of initial PRA and the fall in blood pressure ($r=0.95$; $p<0.001$ at 10 minutes p.i. and $r=0.82$; $p<0.01$ at 10 hours p.i.) when enalaprilic acid was injected alone. When enalaprilic acid was preceded by furosemide, however, no significant correlation was found between the log of the furosemide stimulated PRA and the fall in blood pressure induced by the subsequent injection of enalaprilic acid ($r=0.11$, ns).

Initial blood pressure was not significantly correlated with the fall in blood pressure either after enalaprilic acid alone ($r=0.09$) or after the combination of enalaprilic acid plus furosemide ($r=-0.27$) at any time after injection.

3.2 Effects of enalapril.

3.2.1 Long-term efficacy and safety as compared to conventional treatment.

In this study we compared the effects of long term treatment with enalapril with the effects of conventional treatment in outpatients with essential hypertension. Two treatment protocols were used, described in detail in 2.2.2.1.1. The mild-to-moderate hypertension protocol included patients with a diastolic blood pressure of 96 to 115 mmHg and the moderate-to-severe hypertension protocol included patients with a diastolic blood pressure of 106-115 mmHg.

Patient characteristics are given in table 3.III. Neither in the group of patients with mild to moderate hypertension nor in the group with moderate to severe hypertension there were significant differences in characteristics between the enalapril patients and the propranolol patients. The effects of treatment on blood pressure, heart rate and body weight are given in *figure 3.6.*

Table 3.III Patient characteristics. (mean ±SEM)

	mild to moderate (n=24)		moderate to severe (n=19)	
	enalapril	propranolol	enalapril	propranolol
male/female	4/8	7/8	5/4	3/7
age(years)	47±2	46±2	44±3	47±4
duration(years)	9±2	7±1	8±1	12±2
blood pressure (mmHg)	152/103±3/2	156/101±3/1	168/111±6/2	173/111±8/2
heart rate (bpm)	68±2	70±2	78±6	75±3
body weight(kg)	76.6±3.0	78.1±2.0	75.7±4.0	72.3±3.3
quetelet-index*	258±6	261±11	253±11	264±12
PRA(nmolAI/l/h)	1.0±0.3	1.2±0.4	1.2±0.2	1.2±0.4

*: Quetelet-index is an index for height-adjusted weight; 10^5xweight(kg)/[height(cm)]^2

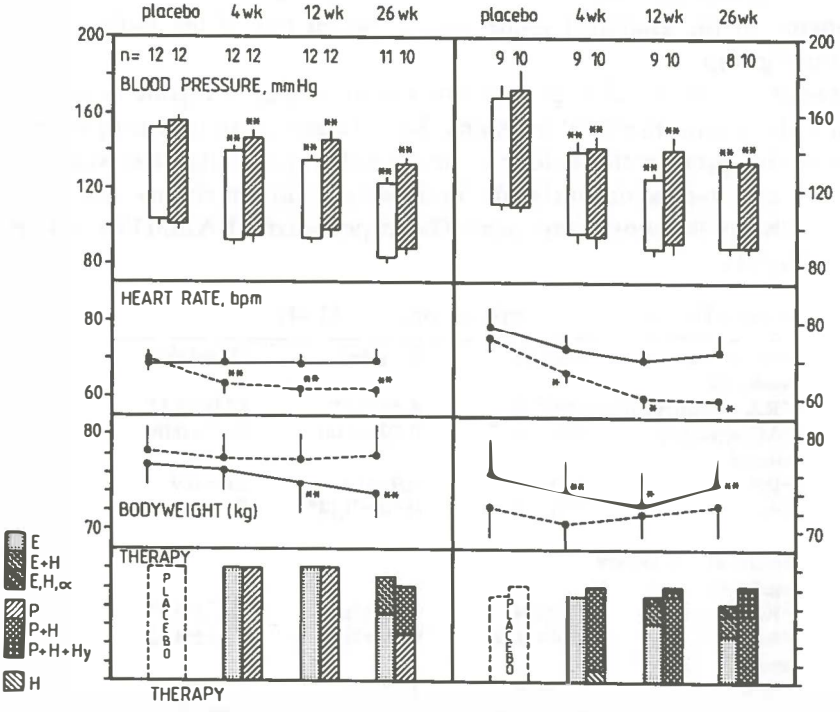


Figure 3.6: *Effects of antihypertensive treatment (mean ± SEM) on blood pressure, heart rate and body weight in the mild to moderate hypertension study (left panel), and the moderate to severe hypertension study (right panel). Data of the patients on enalapril are depicted as open bars (blood pressure) and continuous lines (heart rate and body weight). The bars in the bottom panel depict the dose-distribution.*

In the patients treated with the mild to moderate schedule (left panel), blood pressure decreased with both enalapril and propranolol. The decrease was somewhat more pronounced with enalapril, but the difference between the groups did not reach statistical significance. In the enalapril group the titration criterion (a diastolic blood pressure of 90 mmHg or less) was reached in all patients. Addition of hydrochlorothiazide was required in four patients. In the propranolol group the titration criterion was reached in eight out of twelve patients. Addition of hydrochlorothiazide was required in seven out of twelve patients. Two patients in the control group, in whom diastolic blood pressure remained persistently above 105 mmHg were removed from the protocol as non-responders.

In the patients treated according to the moderate to severe schedule *(figure 3.6,* right panel), the enalapril regimen and the propranolol regimen induced similar decreases. The titration criterion was reached in six out of nine patients in the enalapril group and in seven out of ten patients in the control group.

Heart rate did not change with either of the enalapril regimens, whereas it fell with both propranolol regimens. Body weight fell significantly with both enalapril regimens; this held true for monotherapy enalapril as well as after addition of hydrochlorothiazide. Body weight did not change significantly with either of the control regimens. The responses of PRA and PAC are given in table 3.IV.

Table 3.IV Hormonal response (mean ±SEM)

mild to moderate	placebo	12 weeks	26 weeks
enalapril			
PRA (nmolAI/l/h)	1.0±0.3	8.5±2.8*	12.0±3.1*
PAC(nmol/l)	0.58±0.07	0.39±0.06	0.53±0.09
control			
PRA	1.2±0.4	0.9±0.3	2.0±0.9
PAC	0.74±0.15	0.54±0.14*	0.74±0.14
moderate to severe			
enalapril			
PRA	1.2±0.4	14.2±6.5	16.7±0.9*
PAC	0.54±0.08	0.39±0.06	0.42±0.12
control			
PRA	1.2±0.4	1.2±0.4	1.1±0.3
PAC	0.88±0.19	0.55±0.15*	0.64±0.07

*: $p<0.01$, paired test versus placebo.

With enalapril a sustained rise of PRA was observed with both regimens. The effects on PAC were less consistent; a decrease of borderline

significance is observed after 12 weeks of therapy but after 26 weeks no net change from baseline is found in the enalapril groups as a whole. In the patients on monotherapy enalapril, however, the percentage change in PAC from baseline was -25 ± 19 ($p<0.05$). This response was significantly different ($p<0.05$) from the response in the patients in whom the diuretic was added (+5 ± 11%, NS). With propranolol PAC had fallen significantly after 12 weeks; at 26 weeks the decrease did not reach statistical significance.

Table 3.V Laboraty parameters. (mean ±SEM)

	placebo	12 weeks	%change	26 weeks	%change
		mild to moderate			
enalapril					
Na,mmol/l	141±1	140±1	−1±0*	138±1	−3±1*
K,mmol/l	4.3±0.1	4.5±0.1	ns	4.3±0.1	ns
uric acid,mmol/l	0.33±0.02	0.32±0.01	ns	0.34±0.02	ns
creatinine,umol/l	89±6	86±4	ns	90±6	ns
Hb,gr/l	15.9±0.4	15.4±0.4	ns	15.1±0.4	−8±2*
WBC, 10^6/l	5.7±0.7	6.4±0.7	ns	6.4±0.7	ns
thrombo, 10^6/l	209±13	219±9	ns	225±8	ns
control					
Na	142±1	142±1	ns	140±1	ns
K	4.1±0.1	4.2±0.2	ns	4.0±0.1	ns
uric acid	0.32±0.01	0.34±0.02	ns	0.37±0.02	+5±1*
creatinine	86±4	89±4	ns	85±4	ns
Hb	15.9±0.4	14.7±0.4	−12±2*	15.3±0.4	ns
WBC	5.7±0.4	5.6±0.5	ns	6.0±0.6	ns
thrombo	186±8	182±10	ns	205±5	ns
		moderate to severe			
enalapril					
Na	140±1	140±1	ns	140±1	ns
K	4.3±0.1	4.3±0.1	ns	4.1±0.1	ns
uric acid	0.35±0.01	0.36±0.01	ns	0.35±0.01	ns
creatinine	80±6	84±5	ns	80±5	ns
Hb	15.2±0.5	14.9±0.4	ns	14.5±0.5	ns
WBC	5.5±0.3	5.7±0.4	ns	5.2±0.4	ns
thrombo	216±8	220±6	ns	215±4	ns
control					
Na	141±1	139±1	ns	140±1	ns
K	3.8±0.1	4.0±0.1	ns	4.0±0.2	ns
uric acid	0.32±0.02	0.38±0.03	7±2*	0.38±0.02	6±2*
creatinine	87±6	88±8	ns	91±8	ns
Hb	15.8±0.2	15.7±0.2	ns	15.5±0.4	ns
WBC	5.6±0.6	6.4±0.6	ns	6.6±0.8	ns
thrombo	203±11	211±6	ns	209±4	ns

Laboratory data are given in table 3.V. With the mild to moderate regimen a slight decrease in serum sodium was observed with enalapril at 12 weeks

that became somewhat more pronounced at 26 weeks when hydrochlorothiazide had been added in a subset of the patients. Hb decreased slightly, a finding that was also encountered in the propranolol treated patients. With the moderate to severe regimens, the only consistent change was a rise in uric acid in the patients on the combination of hydrochlorothiazide and propranolol. Other biochemical variables measured included AF, LDH, SGOT, gamma-glutamyltransferase, Ca^{++}, PO_4^-, and serum albumen. These did not change in either of the treatment groups. The differential leucocyte count did not change in either of the treatment groups. Especially no eosinophilia occurred. No abnormalities in the urinary sediment occurred in any of the groups.

One patient presented with atrial flutter after 20 weeks of treatment. At that time he was treated with enalapril 20 mg bid and hydrochlorothiazide 25 mg. His blood pressure was well controlled and serum potassium was normal. Quinidine was added to the medication and sinus rhythm was restored within one week. On the combination with quinidine, orthostatic hypotension occurred that disappeared when hydrochlorothiazide was withdrawn. No cause for the atrial flutter could be found. Otherwise the medication was tolerated well in all patients.

3.2.2 Sodium restriction.

In this study we investigated whether a moderate restriction of dietary sodium potentiates the blood pressure response to enalapril. To this aim ten patients were treated for eight days with enalapril 10 mg bid both during a period on a diet containing 50 and during a period on a diet containing 200 mmol sodium/24h, respectively, in a cross-over fashion. Initial PRA was significantly higher on low sodium (2.3 ± 0.8 nmolAI/l/h versus 1.0 ± 0.3 nmolAI/l/h, $p<0.02$). The initial values of MAP as well as the blood pressure response to treatment are given in *figure 3.7.* for the mean values over the day. It shows that the decrease in blood pressure was slightly but significantly more pronounced on low sodium ($p<0.02$). The reaction to the first dose of enalapril, measured four hours after ingestion is given in *figure 3.8* for both sodium intakes. It shows that in only one patient this initial response was far more pronounced on the low sodium intake. The sharp decrease in blood pressure was well-tolerated in this ambulant patient, without signs of symptomatic hypotension, and no orthostatic hypotension occurred. No significant correlation could be found between the log of initial PRA and the blood pressure response, either for the first-dose effect ($r=0.57$ on liberal sodium and $r=0.14$ on restricted sodium) or for the effect after one week of treatment ($r=0.34$ on liberal sodium and $r=0.46$ on restricted sodium). The effects of enalapril on PRA and PAC are described in detail in chapter 5.

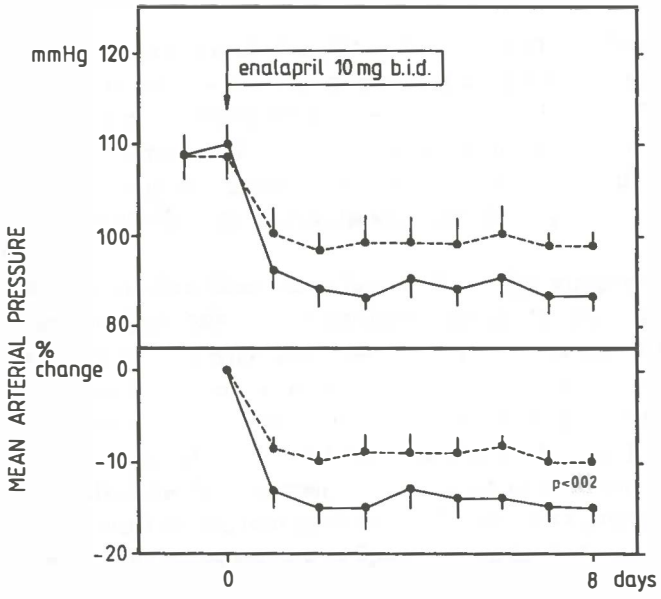


Figure 3.7: The effect of enalapril 10 mg bid on MAP on a 50 mmol (continuous lines) and on a 200 mmol sodium intake (broken lines), as absolute values (upper panel) and as percentage change (lower panel). Blood pressure values were taken as the mean of four values taken over the day. Mean + SEM.

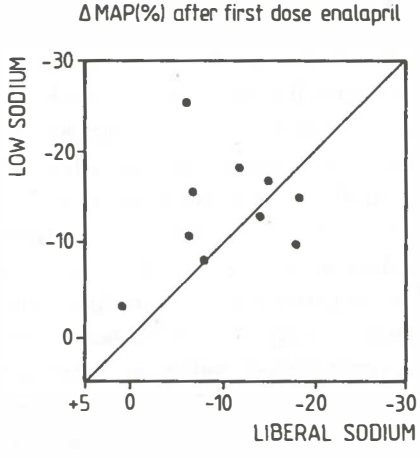


Figure 3.8: Percentage decrease in MAP after the first dose of enalapril on a 50 mmol (Y-axis) as compared to a 200 mmol sodium intake (X-axis).

Discussion

The present study of the effects of enalaprilic acid and enalapril on blood pressure can be divided in three parts. First, the pharmacological studies of the effects of enalaprilic acid, aimed at dose-finding. Second, the therapeutic trials of the efficacy of enalapril in antihypertensive treatment. Finally the studies of the possibility to augment the blood pressure responses to enalaprilic acid as well as enalapril by the manipulation of sodium and renin status.

In the dose-finding study, injection of enalaprilic acid induced a prompt fall in blood pressure on all doses administered in the majority of the patients. Initial PRA rose in the course of this study. This may have influenced our results, as the blood pressure response was correlated with initial PRA. This rise in PRA could be due to a carry-over effect, i.e. accumulation of the drug , persistent drug-induced ACE-inhibition, or to changes in sodium status. The concomitant rise in PAC suggests a decrease in body sodium (due to either better compliance with the diet or to drug-induced sodium loss) rather than accumulation of the drug. As titration was predominantly upward, the rise in PRA could have increased the responses at the higher doses, but as we did not find an augmented response at the higher doses it is not likely our results were influenced this way. One patient in the dose finding group received concomitant therapy. Leaving this patient out of the analysis, however, did not change the shape of the dose distribution curve.

Our titration schedule allowed a dose range of 1.25 to 80 mg. Titration resulted in the administration of doses varying from 5 to 80 mg. This suggest that doses lower than 5 mg are less effective in rapid blood pressure reduction. The up-and-down titration schedule was designed to detect clustering around the optimum dose for rapid blood pressure reduction. It should be stated that this particular study design limits the number of observations on the extremes of the dose range and thus prohibits conclusions as to the shape of the dose-response curve. Nevertheless we found that, within the dose range of 5 to 80 mg, increasing the dose in individual patients did not augment the blood pressure response. The observation that in all patients who received 80 mg, blood pressure was still below baseline 24 hours after injection suggests a more prolonged response at the higher dose. Interpretation of the time course of the response, however, is subjected to yet another restriction. As we titrated at the 10-minute response, slow-responders, if any, may have been overrepresented in the higher doses. With these restrictions in mind our observations are consistent with the statement that once a given dose elicits a blood pressure response of a certain magnitude, increasing the dose not so much augments the response, as well prolongs it. This pattern has been observed with other

ACE inhibitors and may be typical of ACE inhibition (Brunner 1980).

Thus, enalaprilic acid, in doses ranging from 5 to 80 mg rapidly lowered blood pressure in patients with moderate to severe essential hypertension. Therefore, it can probably be added to the available therapeutic armamentarium for those conditions where rapid blood pressure reduction is required and where oral medication is undesirable. We found a considerable variability of the blood pressure response. This finding is consistent with those of other investigators (DiPette 1985). Such a variability is impractical in clinical use. Further studies, therefore, are warranted to define the eventual place of enalaprilic acid.

The efficacy of enalapril in the management of hypertension was investigated in an outpatient study.The patients enrolled had essential hypertension ranging from mild to severe. Enalapril was used as the first step in a stepped-care titration schedule. In this setting, in both mild to moderate and moderate to severe hypertension the enalapril regimens were at least as effective as control treatment, as judged by the decrease in blood pressure. In view of the differences in the titration schedules used, our study design does not allow to draw conclusions as to intrinsic differences in potency between enalapril and either propranolol or hydrochlorothiazide. Strikingly, body weight fell during enalapril treatment as with hydrochlorothiazide, but not with propranolol, a finding also encountered in the multi-center study(Enalapril in Hypertension Study Group 1984). This may have been due to a decrease in caloric intake or, alternatively a diuretic effect of enalapril. The latter possibility finds confirmation in the results described in chapter 5.

The comparative efficacy of the enalapril regimens versus the control regimens was in agreement with the results of the large multicenter trial of which our studies were part. Thus, it appears that enalapril is suitable as a first-line drug in the management of more severe as well as milder forms of essential hypertension. Similar results in mild hypertension have recently been obtained with captopril (Veterans Administration Cooperative Study Group 1983). The efficacy of ACE-inhibitors in milder forms of hypertension was not anticipated at their introduction. Obviously this implies an important enlargement of their therapeutic perspectives.

In our relatively small group of patients enalapril had only few side-effects. In the pooled data of all clinical trials with enalapril (2249 patients), the most frequently reported side-effects were headache(3-4%), dizziness(3-4%) and fatigue(2%). These incidences were not different from the incidences on placebo (McFate Smith 1984). Diarrhoea, nausea and dry cough were reported to occur in 1%. Special attention has been given to the occurrence of rash, taste disturbances, leukopenia and proteinuria, since these have been reported

to occur after captopril. If these side-effects would be due to ACE-inhibition per se, they might seriously limit the use of ACE-inhibitors in clinical practice. With enalapril the overall incidence of rash was only 1%. The incidence of proteinuria (> 1gr/24h) was less than 1% and not different from the incidence with control regimens. Ageusia that could be attributed to the use of enalapril was not reported. Neutropenia has been reported in one patient (Studer 1982), but the relationship to the use of enalapril is doubtful. Thus the incidence of penicillamine-like side-effects is low with enalapril. Yet it is not justified to conclude to a principal difference between enalapril and captopril on these data. The side-effects of captopril are clearly dose-related and occur predominantly in high-risk groups i.e in patients with an impaired renal function and/or auto-immune disease. The experience with enalapril in these groups is still limited.

Of special interest in this respect is the experience in a limited number of patients, intolerant to captopril, who were subsequently instituted on enalapril without reoccurrence of the adverse effect. This has been reported for patients with rash, ageusia, leukopenia, proteinuria and stomatitis (McFate Smith 1984). We have reported on a patient with documented in vivo and in vitro delayed type hypersensitivity to captopril who has been treated effectively with enalapril for over two years without evidence of in vivo or in vitro cross-rectivity (Navis 1984).

After captopril first-dose hypotension has been reported frequently, especially in patients who were sodium-depleted and in patients with heart failure (Hodsman 1983, Vidt 1982). This adverse effect is thought to be pharmacological, i.e. due to ACE-inhibition per se. We did not observe first-dose hypotension after enalapril. It should be noted, however, that our patients had uncomplicated essential hypertension, and none of them was sodium-depleted. Indeed, hypotension occurs occassionally after enalapril in patients with hypertension (McFate Smith 1984). In these patients sodium status was not documented. Hypotensive reactions, in association with bradycardia, have also been reported after enalapril in patients with congestive heart failure (Cleland 1985).

After acute ACE-inhibition with captopril or teprotide the fall in blood pressure has been found to be more pronounced in patients with high initial PRA (Bravo 1979, Brunner 1980, Case 1977). Moreover, on maintenance treatment with ACE-inhibition the blood pressure response is potentiated by the addition of diuretics as well as by dietary sodium restriction. Thus the magnitude of the fall in blood pressure induced by ACE-inhibitors is related to the prevailing state of activation of the RAAS. From these data we expected that the fall in blood pressure after enalaprilic acid as well as enalapril

would be correlated to the log of initial PRA. Furthermore, we expected that it would be possible to potentiate the blood pressure response to enalaprilic acid as well as enalapril by preceding stimulation of the RAAS.

Indeed, we found after enalaprilic acid a correlation between the fall in blood pressure and the log of initial PRA on each of the sodium intakes studied. No such correlation was found, however, after treatment with enalapril. Of the ACE-inhibitors captopril, teprotide, enalaprilic acid and enalapril, the latter has the slowest onset of action. Presumably the rapid onset of action of enalaprilic acid allows a more precise determination of the relationships between circulating PRA and blood pressure.

Restriction of dietary sodium led to an increase in PRA and to a potentiation, albeit slight, of the responses to both enalaprilic acid and enalapril. When renin stimulation was induced by injection of furosemide, however, no potentiation of the blood pressure response was observed. This was true for the response immediately after injection as well as the responses at the moment of maximum sodium loss. This lack of potentiation contrasts with reports on the potentiation by diuretic treatment and sodium depletion of the blood pressure response to both captopril and enalapril (Bravo 1979, Ferguson 1982). Recently however, Kelly and co-workers found that furosemide failed to potentiate the fall in blood pressure after captopril (Kelly 1983). How could furosemide stimulate PRA and yet not potentiate the blood pressure response to ACE-inhibition? It could be that direct vascular effects of furosemide (Hesse 1975) or reflex sympathetic activation offset a more pronounced fall in blood pressure. It could also be that PRA immediately after furosemide reflects an intrarenal effect of furosemide rather than the contribution of the RAAS to the maintenance of blood pressure (Vander 1969).

Thus, in our patients a moderate restriction of dietary sodium potentiated the blood pressure response to enalaprilic acid as well as enalapril, without inducing symptomatic hypotension at the onset of treatment. Obviously, the therapeutic implication of this observation is that sodium restriction increases the efficacy of antihypertensive treatment with enalapril. This observation has een made with captopril also (Bravo 1979), and it has been interpreted as evidence for the important role of interference with the RAAS as mechanism of action of these ACE-inhibitors (Rubin 1980). Sodium restriction, however, has been known to increase the efficacy of various other types of antihypertensives also (Dustan 1974, 1983, Gifford 1984). In addition, it should be noted that for a given sodium intake as well as a given PRA-level the interindividual variability in the response to enalaprilic acid as well as enalapril was considerable. In particular, this variability was larger than the potentiation that could be induced by moderate sodium restriction. This

suggests that characteristics other than sodium intake and renin status also determine the responsiveness to ACE-inhibition.

Thus, in our studies as well as in the work of others, enalapril appears an effective drug with few side-effects when used as a first-line drug in uncomplicated essential hypertension. Enalaprilic acid also effectively lowers blood pressure but further studies are required to define its place in therapy. Finally, a few remarks should be made about the use of enalapril in clinical practice. In our outpatient studies enalapril was administered twice daily, in doses ranging from 5 mg bid to 20 mg bid. It has been shown, however, as could be anticipated from the pharmacokinetic properties, that once daily dosing is as effective as twice daily dosing (Bergstrand 1982). Therefore, in hypertension the recommended dose is 10 mg oid, which can be increased to 20 mg oid. In patients with a creatinine clearance below 30 ml/min the starting dose should be 5 mg, or even 2.5 mg.

Consistent with the findings of other investigators with captopril we found that sodium restriction potentiates the blood pressure response to enalapril. Consequently, if blood pressure fails to normalize on monotherapy enalapril restiction of dietary sodium restriction is the most logical next step to augment its efficacy. If the therapeutic gain of sodium restriction is insufficient, a diuretic should be added. On the other hand the blood pressure response to enalapril (and to ACE-inhibitors in general) in patients already using a diuretic is difficult to predict and may be pronounced. Therefore sodium balance should preferably be restored by withdrawal of the diuretic three days before instituting therapy with an ACE-inhibitor.

CHAPTER 4. RENAL FUNCTION

Introduction.

This chapter deals with the effects of enalaprilic acid and enalapril on renal hemodynamics. It has consistently been shown that ACE-inhibition induces renal vasodilatation. ERPF has been reported to rise after ACE-inhibition in animals; in normal subjects and in patients with hypertension. GFR has been reported to rise also, to remain unchanged or to fall (Hollenberg 1977, 1979, 1981, Kimbrough 1977, de Leeuw 1983, Zimmerman 1981). Filtration fraction invariably falls. We studied the renal hemodynamic effects of enalaprilic acid and enalapril in hypertensive patients with special reference to their specificity for ACE-inhibition and, more specifically, for interference with the RAAS. With respect to this question different approaches were followed.

We studied the acute effects of enalaprilic acid on renal hemodynamics and investigated whether these could be abolished by intravenous infusion of exogenous AII.

As to the renal effects of enalapril in hypertensive patients we investigated whether these were different from those elicited by conventional therapy.

Furthermore, we investigated whether the renal response to enalapril depends on sodium status and the concomitant state of activation of the RAAS. To this aim we studied renal hemodynamics in hypertensive patients on a liberal sodium diet and on a moderately restricted sodium diet, before and after one week of treatment with enalapril.

4.1 Effects of enalaprilic acid.

4.1.1 Acute effects on renal function.

The acute effects of enalaprilic acid on renal function were studied in 14 patients. The protocols are described in detail in 2.2.1.2. In 6 out of 14 patients we also studied the effects of placebo to account for non-drug-specific effects.

After the effects of enalaprilic acid on renal hemodynamics and sodium excretion had been established, another experiment was carried out in five patients. After injection of enalaprilic acid a graded infusion of AII was given in an attempt to abolish the renal effects of enalaprilic acid. The effects on renal hemodynamics will be described in the present chapter, the effects on urinary electrolyte excretion will be dealt with in chapter 5.

Table 4.I Initial values (means±SEM, n=14)

GFR(ml/min/1.73m²)	96±4
ERPF(ml/min/1.73m²)	363±22
FF	0.26±0.01
PRA(nmolAI/l/h)	0.8±0.2

The initial values of the 14 patients are given in table 4.I. In three patients renal function was slightly impaired with a GFR of 55, 74 and 78 ml/min, respectively.

The effects of placebo and of enalaprilic acid on renal hemodynamics. are shown in *figure 4.1.* It shows, that injection of placebo did not result in consistent changes in renal hemodynamics.

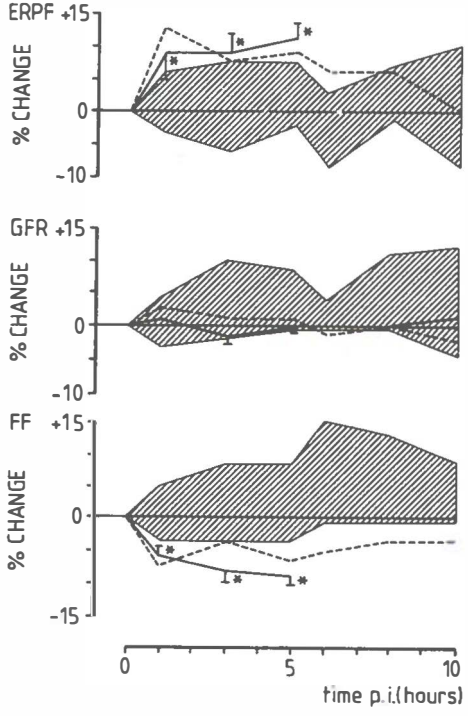


Figure 4.1:
Effects of enalaprilic acid on ERPF, GFR and FF up to five hours after injection (n=14, mean ± SEM, continuous lines), and up to ten hours after injection (n=5, median, broken lines). The shaded area depicts the range of the values measured after placebo (n=6).: p<0.01, paired test versus baseline value.*

Injection of enalaprilic acid was followed by an increase in ERPF of 9 ± 4 per cent ($p<0.01$) in the first hour. This response was sustained up to at least five hours after injection. In the five patients in whom renal function was measured up to ten hours after injection, a gradual return to baseline was observed five to ten hours after injection. Mean GFR did not change at any time during the observation period. FF fell by 6 ± 2 per cent ($p<0.01$) in the first hour and gradually further decreased up to five hours after injection.

The percentage change in ERPF was positively correlated with log of initial PRA ($r=0.66$; $p<0.01$) and inversely with the percentage change in MAP ($r=-0.79$; $p<0.001$) and with age ($r=-0.69$: $p<0.001$). The percentage change in GFR was not significantly correlated with either the log of initial PRA ($r=0.35$) or the percentage change in MAP ($r=-0.36$). It was inversely correlated with age ($r=-0.63$; $p<0.01$) and positively with the change in ERPF ($r=0.84$; $p<0.001$) as well as the percentage change of the product of MAP and ERPF ($r=0.63$; $p<0.01$).

Injection of placebo led to a slight fall in blood pressure in all six patients in the first hour after injection. The median change in mean arterial pressure was −3 per cent (range −2 to −8) ($p<0.05$). After the first hour no more consistent changes were noted: 0 (−2 to +12) and 0 (−4 to +6) per cent at five and ten hours, respectively. Injection of enalaprilic acid led to a prompt fall in blood pressure of 9 ± 2 per cent after one hour ($p<0.01$) that was sustained during the whole observation period. The magnitude of the blood pressure response was similar after the 5 mg and after the 10 mg dose. The percentage change in MAP after enalaprilic acid was inversely correlated with the log of initial PRA ($r=-0.59$, $p<0.05$).

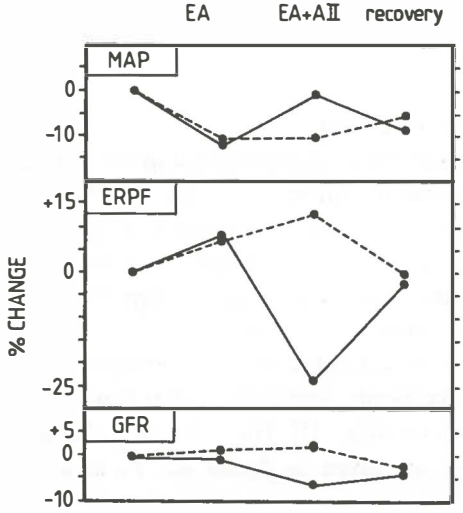


Figure 4.2:
Percentage change (median) in MAP, ERPF and GFR after enalaprilic acid (EA) alone (control day, broken lines) and after enalaprilic acid followed by AII-infusion (AII day, continuous lines).

4.1.2 Effects of AII on the renal response to enalaprilic acid.

In five patients, all with GFR 90 > ml/min, the effects of a graded infusion of AII were studied after injection of enalaprilic acid. *Figure 4.2* gives the effects of the injection of enalaprilic acid on blood pressure and renal hemodynamics with and without a subsequent infusion of AII. It shows that, until AII is infused, the response to the respective enalaprilic acid injections is fairly similar, both for blood pressure and renal function. To titrate blood pressure back to baseline values, AII was infused in doses ranging from 2.8 to 31.9 ng/kg/min. The dose of AII required to bring blood pressure back to baseline was not related to the fall in blood pressure after enalaprilic acid. The doses that brought blood pressure back to baseline (with a range of -4 to + 10%) led to a sharp reduction of ERPF to values of 19 to 36 per cent below baseline. The fall in GFR, also to below baseline values, was less pronounced.

After a recovery of two hours, both ERPF and GFR had returned to values that were not significantly different from those after the control injection of enalaprilic acid. The decrease in ERPF after AII was most pronounced in the patients that received the highest dose of AII ($r=-0.95$; $p<0.01$). An inverse correlation was found between the decrease in ERPF expressed as percentage change from baseline after AII, and the increase in ERPF found after the control injection of enalaprilic acid ($r=-0.76$; $p<0.05$), i.e. the patients with the most pronounced increase after ACE-inhibition also exhibited the greatest decrease after AII-infusion. Such a relationship could not be found for GFR. The responsiveness of ERPF and GFR to AII could not be related to the level of initial PRA.

4.2 Effects of enalapril.

4.2.1 Long term effects of antihypertensive treatment.

The long term effects of antihypertensive treatment with enalapril on renal hemodynamics were compared with those of conventional therapy. To this aim renal function studies were performed in the patients participating in the two double-blind protocols on antihypertensive treatment: the mild-to-moderate hypertension protocol and the moderate-to-severe hypertension protocol. These protocols are described in detail in 2.2.2.1.1 and in 2.2.2.1.2. A total number of 41 patients was studied after four weeks of treatment, 41 patients were studied after 12 weeks of treatment and 35 patients were studied after 26 weeks of treatment. Patient characteristics are given in table 3.III. The individual changes from baseline for ERPF, GFR and FF are given in *figures 4.3, 4.4 and 4.5.*

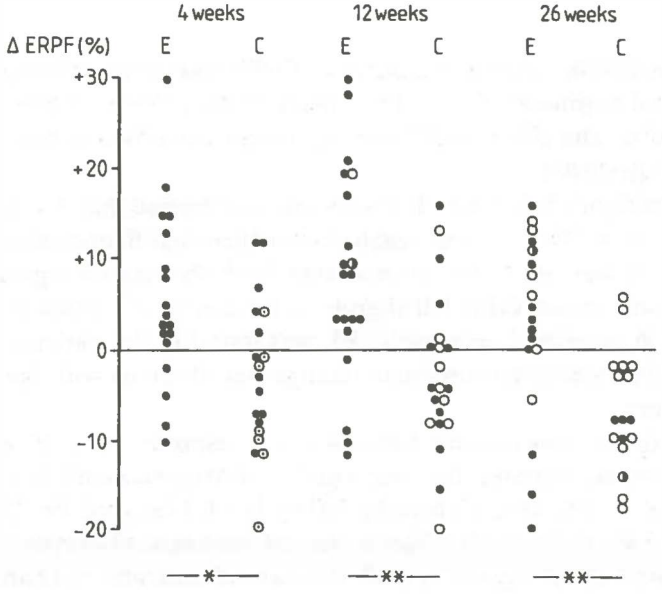


Figure 4.3: Percentage change in ERPF after four, twelve and twenty-six weeks of therapy with the enalapril regimens (E) and with the control regimens (C). Closed circles depict the patients on monotherapy enalapril or propranolol, dotted circles depict the patients on monotherapy hydrochlorothiazide, and the open circles depict patients on the combination of either enalapril or propranolol with hydrochlorothiazide.

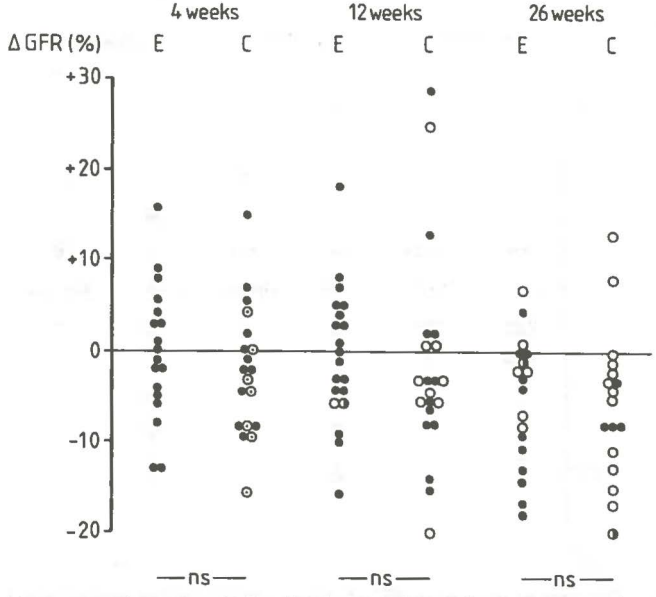


Figure 4.4: Percentage change in GFR after four, twelve and twenty-six weeks of treatment with the enalapril regimens (E) and the control regimens (C). Symbols: see 4.3.

It appears that the effect of enalapril on ERPF was different from the effect of the control regimen($p<0.01$). The effects of the different regimens on GFR were similar. The effects on FF were again significantly different between the regimens ($p<0.01$).

With enalapril ERPF rose in the majority of the patients in spite of a fall in blood pressure. The increase reached statistical significance after 4($p<0.02$), and after 12 weeks($p<0.01$) of treatment. With the control regimen no such increase was found. GFR fell slightly in the course of treatment with either regimen. A persistent decrease in FF was found in the patients on enalapril($p<0.01$) whereas no consistent change was observed with the conventional regimen.

These differences in renal hemodynamic response occurred in spite of a blood pressure response that was similar: during enalapril blood pressure (initial value: 159/106±4/2 mmHg) fell by 11±1, 13±2 and 19±2% after 4, 12 and 26 weeks, respectively. During control treatment blood pressure (initial value: 164±4/2 mmHg) fell by 8±2, 12±2 and 15±2% after 4, 12 and 26 weeks, respectively.

It can be appreciated from *figures 4.3, 4.4 and 4.5* that there was a considerable interindividual variability in the renal hemodynamic response to both the enalapril regimens and the control regimens. As to enalapril treatment, no difference in response was found between the patients on monotherapy

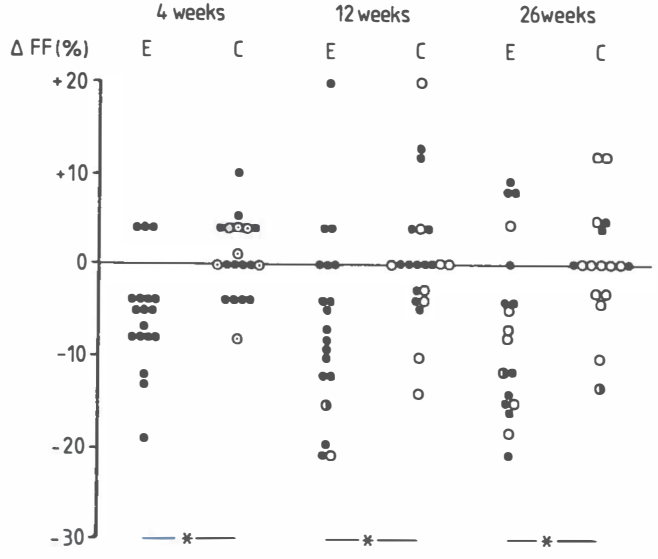


Figure 4.5: Percentage change in FF after four, twelve and twenty-six weeks of treatment with the enalapril regimens (E) and with the control regimens (C). Symbols: see 4.3.

enalapril and the patients in whom hydrochlorothiazide was added. We found a weak negative correlation between initial ERPF and the subsequent response to enalapril($r=-0.45$, $p<0.05$), implying that increases in ERPF were predominantly observed in patients in whom ERPF was relatively low at the onset of treatment. A similar correlation was found for GFR ($r=-0.74$, $p<0.01$). No such correlations were found with control treatment.

In the patients on enalapril the change in blood pressure correlated weakly and negatively with the change in ERPF($r=-0.54$, $p<0.05$) but not with the change in GFR, implying a greater increase in ERPF in the patients with the largest drop in blood pressure. With propranolol the change in blood pressure was weakly and positively correlated with the change in GFR($r=0.53$, $p<0.05$), but not with the change in ERPF($r=0.32$,ns), implying a greater fall in GFR in the patients with the largest drop in blood pressure. In neither group the responses of blood pressure and renal hemodynamics were correlated with initial PRA.

4.2.2 Sodium restriction.

The influence of sodium intake on renal hemodynamics and on the renal response to enalapril was studied in nine patients with strictly normal renal function (i.e. a GFR greater than 90 ml/min). In these patients renal function studies were performed on a liberal(200 mmolNa^+/24h) and on a moderately restricted(50 mmolNa^+/24h) sodium intake, before and after one week of treatment with enalapril. The protocol is described in detail in 2.2.2.2.2.

Table 4.II Baseline values. (n=9, mean ± SEM)

	liberal sodium	low sodium	%change	p
MAP (mmHg)	109±2	110±2	+1±2	ns
GFR(ml/min/1.73m²)	111±4	104±4	−7±2	0.02
ERPF(ml/min/1.73m²)	431±22	415±27	−4±3	0.1
FF	0.26±0.01	0.26±0.01	+1±3	ns

The baseline values on low and liberal sodium are given in Table 4.II. Blood pressure was similar on both sodium intakes. GFR was significantly lower on low sodium. In seven out of nine patients ERPF was lower also. The individual values for GFR and ERPF on low and liberal sodium are depicted in *figure 4.6.*

Blood pressure fell after enalapril on both sodium intakes; the decrease was slightly but significantly more pronounced during the low sodium diet (3.2.2). The renal hemodynamic response to enalapril on both sodium intakes is given is *figure 4.7.* GFR increased after enalapril on low

sodium despite the fall in blood pressure. On the liberal sodium intake, however, no change was observed. ERPF increased on both intakes; the increase was more pronounced on the low sodium diet ($p<0.02$). Filtration fraction decreased similarly on both sodium intakes.

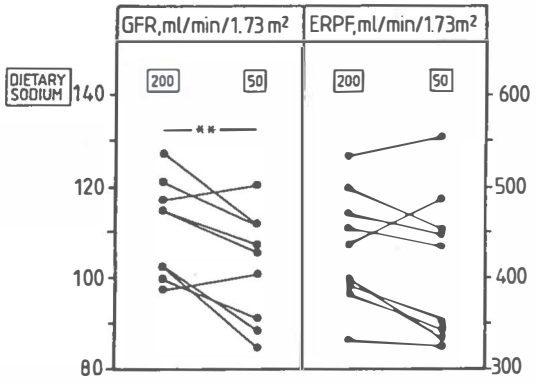
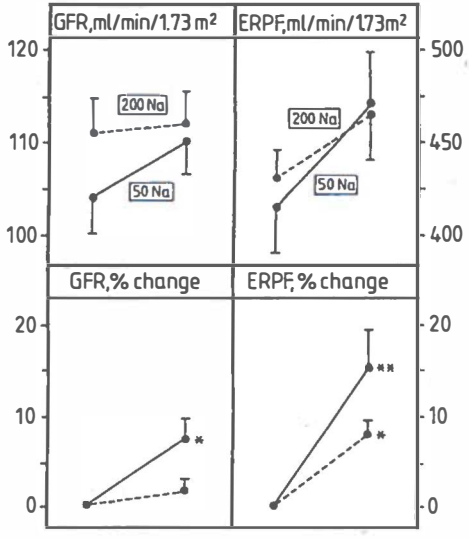


*Figure 4.6: Individual values of GFR and ERPF on liberal and low sodium intake. **:$p<0.02$.*



EFFECTS OF ENALAPRIL

*Figure 4.7: Effects of enalapril (mean ± SEM) on GFR and ERPF on liberal (broken lines) and on low sodium (continuous lines). Data are given as absolute values (upper panel) and as percentage change (lower panel). *:$p<0.02$; **:$p<0.01$.*

Thus the renal response to enalapril was significantly different on the different sodium intakes. To determine how this difference was related to the difference in baseline values we also calculated both responses as the percentage change from the same baseline, i.e. the initial value on liberal sodium intake. This is depicted in *figure 4.8.* It shows that, once the same reference value is chosen, the responses of both GFR and ERPF are virtually similar on low and on liberal sodium intake.

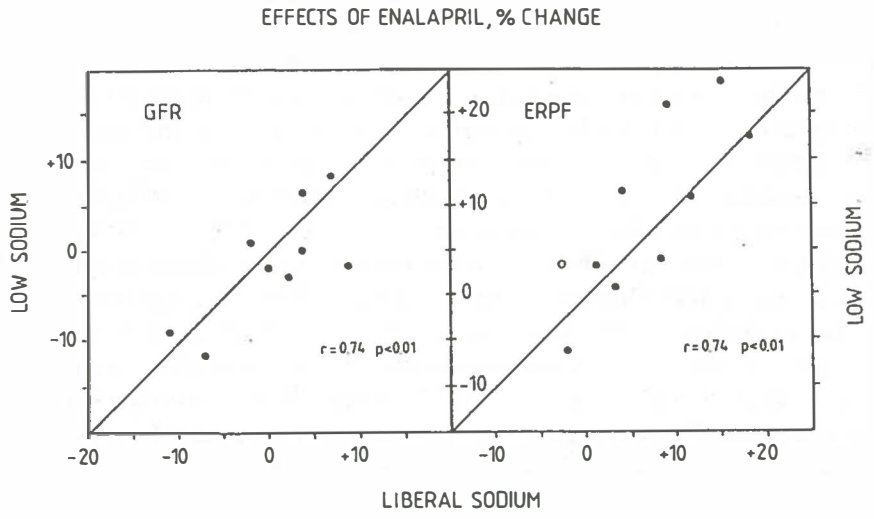


Figure 4.8: *Effects of enalapril on GFR and ERPF during liberal (X-axis) as compared to low (Y-axis) sodium diet. Data are given as the percentage change from the same reference value, i.e the baseline value on liberal sodium, and plotted out with the line of identity.*

Discussion.

This chapter describes the effects of enalaprilic acid and enalapril on renal hemodynamics. These effects were studied with special reference to their specificity for ACE-inhibition. Enalaprilic acid led to an increase in ERPF within an hour after injection. Despite a fall in blood pressure GFR did not change. Consequently FF fell. No such changes occurred after placebo, so they can be considered drug-specific. A similar acute increase in ERPF has been observed after teprotide and captopril, both in normal and hypertensive

subjects and in animals (Hollenberg 1977, 1979, 1981, Kimbrough 1977, Zimmerman 1981), suggesting this increase is specific for ACE-inhibition. GFR has been reported to rise or to remain unchanged after ACE-inhibition, at least in the absence of renal artery stenosis or severe volume depletion.

The increase in ERPF without change in GFR leading to a fall in FF, is consistent with renal vasodilation located at the level of the efferent arteriole. As AII exerts its renal vasoconstrictive effect mainly on the efferent arteriole (Edwards 1983, Steinhausen 1983), such a vasodilation could well be explained by a decreased formation of AII. A redistribution of renal blood flow to the more superficial cortical nephrons (that generally have a lower FF than the juxtamedullary nephrons) can also be expected to lead to a fall in FF. Our study does not allow, however, a conclusion as to the exact intrarenal mechanism of the renal hemodynamic response to enalaprilic acid.

Acute changes in GFR induced by antihypertensive treatment are known to be related to the changes in both ERPF and MAP (Reubi 1978). The positive correlation we found in our patients between the change in GFR and the change in the product of ERPF and MAP indicates the relationship applied to our patients also. Thus, the increase in ERPF allows to compensate for the fall in blood pressure. This response was observed to be more adequate in the younger patients. Similar observations have been made after captopril as well as after other renal vasodilators (Hollenberg 1974a, Hoorntje 1981). The less pronounced renal vasodilation in older patients probably reflects the presence of fixed organic lesions (Hollenberg 1974a), leading to decreased renal reserve capacity (ter Wee, 1986)

To further define the role of decreased formation of AII, we investigated whether the renal response to enalaprilic acid could be reversed by infusion of exogenous AII. By graded infusion we could titrate blood pressure back to baseline. Concomitantly, ERPF and GFR decreased to values below baseline. Thus, although there was a quantitative discrepancy in the responses of systemic and renal hemodynamics, the renal response to enalaprilic acid was abolished by AII indeed.

Interestingly, the patients with the smallest increase in ERPF (as a percentage from baseline) after enalaprilic acid also had the smallest decrease after intravenous exogenous AII (again as a percentage from baseline). If the latter implies a relative insensitivity to circulating AII (for instance by high intrarenal AII levels), the smaller response to enalaprilic acid would imply that this response is mainly mediated -at least in this acute situation- by a decrease in circulating AII levels rather than by a decrease in intrarenal AII. This is consistent with recent findings in the dog, demonstrating that the effects of enalaprilic acid on renal hemodynamics are much more pronounced when it

is administered intravenously than when it it injected directly into the renal artery (Zimmerman 1985). Caution is warranted in interpreting the data of the AII infusion however, as our patients received different doses AII and the response to AII was clearly dose-related.

The effects of maintenance treatment with enalapril on renal hemodynamics were assessed in the patients treated according to the mild-to-moderate hypertension protocol as well as the moderate-to-severe hypertension protocol. The medication was titrated on the blood pressure response, and additional medication was given if required. Therefore, this study does not allow conclusions as to the pharmacological effects of enalapril, propranolol or hydrochlorothiazide per se. It does allow, however, a comparison of the overall effects on renal hemodynamics of enalapril treatment in the context of an antihypertensive regimen, with control treatment consisting of a conventional stepped care regimen. As such, it allows an assessment of the clinical relevance of the pharmacological effects of ACE-inhibitors on renal function in clinical practice.

The pattern that has been described as typical for ACE-inhibition, i.e. an increase in ERPF, unchanged GFR and a fall in FF (Zimmerman 1981), indeed was encountered in our patients on enalapril during the first three months of treatment. No such changes were found with control treatment, thus our findings provide additional evidence for the specificity of this renal hemodynamic response for ACE-inhibition. On prolonged treatment, however, the increase in ERPF with enalapril tended to disappear, whereas GFR fell somewhat on both regimens. Thus, the responses to the two regimens were not constant over time. This has also been described in studies with other antihypertensive regimens, and has been attributed to homeostatic readjustment of renal vascular tone (Glück 1984, Reubi 1970). Yet a difference in renal hemodynamic profile between the two regimens persisted throughout the study period, as a persistent fall in FF was observed with enalapril whereas no such decrease occurred with the control regimen. Patients with established essential hypertension are known to have relatively high FF (Schalekamp 1970). The fall in FF after ACE-inhibition can be interpreted as a correction of this abnormality. Whether this renal hemodynamic response indeed constitutes a benefit for the hypertensive patient, however, cannot be deduced from the present study.

The responses of both ERPF and GFR to enalapril were negatively correlated with their initial values, indicating better preservation of renal function in the patients in whom it was impaired at the onset of treatment. This pattern was not encountered with the control regimens. Since it has been observed after captopril also, it may be specific for ACE-inhibition (Hollenberg 1979).

This finding is thought to be due to a relatively high AII-mediated renal vascular tone in the subjects with the lowest values for ERPF and GFR at the onset of treatment (Meggs 1980).

The response of ERPF to enalapril was negatively, albeit weakly, correlated with the blood pressure response. This may indicate that the blood pressure response and the renal response to enalapril are to a certain extent mediated by a common mechanism. Alternatively, one might speculate renal effects contribute to the lowering of blood pressure (Antonaccio 1979, Meggs 1980). Again, this pattern is different from the findings with the control regimens. With control treatment a greater decrease in blood pressure is associated with a more pronounced decline in GFR, suggesting that in these patients renal autoregulation insufficiently compensates for the fall in blood ressure.

On long-term treatment GFR fell slightly with both enalapril and control treatment. This is somewhat disappointing as one of the major aims of treating high blood pressure is to prevent a decline in target organ function. A relatively large proportion of our patients however, had mild to moderate hypertension, and it may be that follow-up has to be much longer to disclose benefit, if any, in this category of patients.

We finally studied the effects of enalapril on renal function on low as compared to liberal sodium diet to assess whether the renal response to enalapril depends on sodium status and the concomitant state of activation of the RAAS.

For the study of this question it is relevant that alterations in sodium status itself can influence renal hemodynamics. It has long been known, that a restriction of dietary sodium to less than 10 mmol/24 h (i.e. to less than 5 per cent of the habitual intake) results in a decrease in GFR and ERPF in hypertensive patients (Chasis 1950) and in healthy individuals (Romero 1968). Our results show that a moderate decrease to approximately 25 per cent of the habitual intake is already associated with a fall in GFR and, albeit less consistently, ERPF.

The effects of enalapril on renal hemodynamics were significantly more pronounced on low sodium intake. This underlines once more the importance of sodium status when considering the effects of ACE-inhibition. It could well be that a large part of the divergence in the data on the effects of ACE-inhibitors on GFR is accounted for by differences in the sodium status in the studies involved. Our findings are in accord with a variety of acute experiments in animals (Hall 1980, Kimbrough 1977, Mimran 1974) and man (Hollenberg 1981) in which salt-loading blunted or abolished the increase in renal blood flow observed after ACE-inhibition or after an AII-

antagonist. This has provided evidence that the RAAS, probably AII-mediated vasoconstriction, is involved in the renal hemodynamic response to sodium restriction.Yet it has recently been called doubtful that the renal hemodynamic response is only accounted for by the RAAS, in view of the quite pronounced changes in the renin-system as compared to the subtle changes in renal function (Zusman 1984).

What insights could this study provide as to the response of renal function to differences in sodium intake? To our knowledge, it is the first study thus far with the individual subjects as their own control, thus allowing a strict comparison of the responses to ACE-inhibition on the different sodium intakes. The blood pressure response to sodium restriction is known to be highly variable among different individuals (Kawasaki 1978, Luft 1982). Therefore, in any study on the effects of sodium restriction the individual responses are important. On one hand, the blood pressure response could affect renal hemodynamics, on the other hand, the renal hemodynamic response may be part of the more or less effective homeostatic response aimed at keeping blood pressure constant. In our patients, ACE-inhibition restored ERPF on the low sodium diet to precisely the level measured on liberal sodium intake. Similarly, ACE-inhibition restored GFR on low sodium intake to a level only insignificantly below that on liberal sodium. As a change in GFR is related to the change in ERPF as well as the change in blood pressure (Reubi 1978), the less precise match of GFR may be due to the somewhat more pronounced fall in blood pressure on the low sodium diet. In agreement with the above-cited studies, our results strongly suggest that the lower values of GFR and ERPF on low sodium diet are due to an AII-mediated renal vasoconstriction, although they do not rule out the possibility of a role for other hormonal systems. It should be noted, that our study was the only one with an only moderate restriction of dietary sodium, and that our results were obtained in the absence of excessive activation of the RAAS. This may imply that the role of the RAAS in the modulation of renal hemodynamics in response to altered sodium status is not confined to situations of severe sodium depletion, but also active over a range of relatively normal states of sodium balance.

Thus we found that both the active metabolite enalaprilic acid and the parent compound enalapril induce a distinct renal response that, in view of its similarity to that of other ACE-inhibitors, probably is specific for ACE-inhibition. The renal response depends on both sodium-renin status and the time course studied. Although not necessarily conclusive, both the potentiation of the response by sodium restriction and the annihilation of the acute response by AII point to the importance of interference with the RAAS as a

mechanism of action. On long term treatment the renal response to ACE-inhibition becomes less distinct, albeit still different from control treatment. Whether the remaining difference in renal hemodynamics constitutes an additional benefit over the antihypertensive efficacy remains to be investigated.

CHAPTER 5 SODIUM EXCRETION.

Introduction.

The importance of body sodium status in high blood pressure and in antihypertensive treatment has long been recognized (Borst 1963, Freis 1979, Guyton 1974). In spite of widespread interest in the renal effects of ACE-inhibitors, their effects on sodium excretion in antihypertensive treatment are not well defined. In acute experiments both increased and unchanged sodium excretion have been reported after captopril as well as enalapril (Bengis 1981, Hollenberg 1981, McCaa 1978, Tarazi 1980, Zimmerman 1981). Whether continued treatment induces net sodium loss from the body is also controversial. Both captopril and enalapril were reported to induce negative sodium balance (Atlas 1979, de Leeuw 1983, MacGregor 1981); other investigators, however, did not confirm this finding (Hodsman 1984, Johns 1980). Both sodium status and renin status have been shown to influence the effects of ACE-inhibitors and may account for some of the disparity in the observations thus far. In the present study, therefore, we investigated first, whether enalaprilic acid can promote sodium excretion, second, whether continued treatment with enalapril indeed induces net sodium loss from the body and finally, whether the effects of enalapril on sodium balance are influenced by the prevalent sodium intake.

5.1 Enalaprilic acid.

5.1.1 Acute effects on sodium excretion.

Fourteen patients, in balance on a 100 mmol sodium intake, were studied. During the experimental days the patients remained recumbent. They received an injection of enalaprilic acid (10 mg, n = 11; 5 mg, n = 3) or placebo at noon. Electrolyte excretion and blood pressure were measured from 8 am to 10 pm; renal function was monitored from 8 am to either 5 pm or 10 pm. Details of the protocols have been given in 2.2.1.2.1.

The initial values of the patients are given in Table 5.I. No consistent changes in sodium excretion were observed after placebo. Median changes from baseline were -19(-43 to -5) per cent and -7(-31 to +300) per cent after one and five hours, respectively. For potassium excretion these values were -5(-11 to+38) per cent and -16(-43 to -7) per cent respectively. Phosphate excretion increased by +33(+20 to +300) per cent ($p<0.01$) and +100(+14 to +1050) per cent ($p<0.01$), respectively.

Table 5.I Initial values
(n=14, mean ± SEM)

MAP(mmHg)	124±2
GFR(ml/min/1.73m²)	96±4
UNaV(mmol/h)	4.6±1.7
PRA(nmolAI/l/h)	0.8±0.2

The effects of enalaprilic acid on the excretion of sodium and potassium are shown in *figure 5.1.* Baseline sodium excretion was 4.6 ± 1.7 mmol/h. Sodium excretion increased within the first hour after injection in eleven out of fourteen patients and somewhat later, i.e. after four to five hours, in two of the remaining patients. The mean increase in sodium excretion after five hours was 61 ± 17 per cent ($p<0.01$). This increase was sustained during the whole observation period of 10 hours. Potassium excretion was significantly

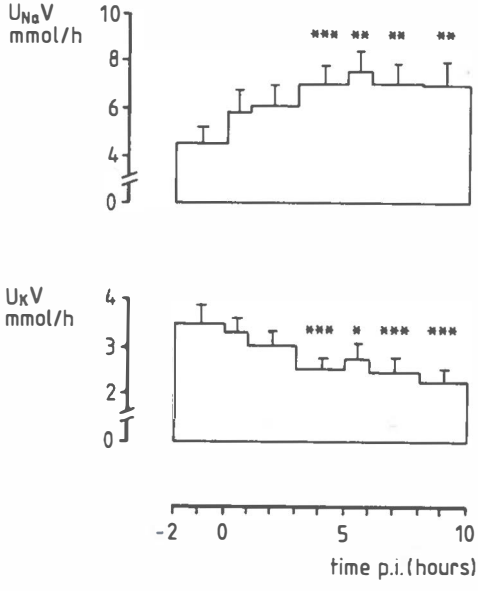


Figure 5.1:
Effects of enalaprilic acid on the urinary excretion of sodium (UNaV) and potassium (UKV). Mean ± SEM.
**:p<0.05; **:p<0.02;*
****p<0.01, paired test versus baseline.*

below baseline from four to five hours after injection ($p<0.01$). Consequently, the sodium/potassium ratio increased. Phosphate excretion increased significantly by 96 ± 17 per cent ($p<0.01$) after one, and by 219 ± 46 per cent ($p<0.01$) after five hours and remained so during the hole observation period.

The percentage increase in sodium excretion was positively correlated with log initial PRA ($r=0.65$; $p<0.01$) *(figure 5.2)* and negatively with the percentage change in PAC ($r=-0.56$; $p<0.05$). The percentage increase in phosphate excretion was correlated with both log initial PRA ($r=0.71$; $p<0.01$) and with the percentage increase in sodium excretion ($r=0.69$; $p<0.01$). The change in potassium excretion was not significantly correlated with log initial PRA ($r=-0.41$; ns).

Mean arterial pressure fell after enalaprilic acid in all patients within an hour after injection (mean decrease -9 ± 2 per cent, $p<0.01$). The decrease persisted throughout the observation period. ERPF increased by 9 ± 4 per cent ($p<0.01$) and GFR did not change. The effects of enalaprilic acid on blood pressure and renal hemodynamics have been given more extensively in 4.1.1.

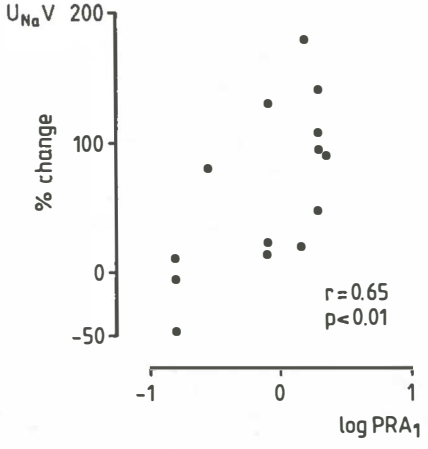


Figure 5.2:
Correlation between the percentage increase in urinary sodium excretion after enalaprilic acid and the log of initial PRA.

5.1.2 Effects of AII on the effects of enalaprilic acid on electrolyte excretion.

The effects of a graded infusion of exogenous AII on the effects of enalaprilic acid on electrolyte excretion were studied in five patients.In these patients the effects of 10 mg enalaprilic acid on electrolyte excretion, renal hemodynamics and blood pressure were established first. Then, in a second experiment, after at least three days wash-out, another injection of enalaprilic acid was given. In this second experiment, a graded intravenous infusion of AII was started at the moment the renal response was known to level off at its

maximum. The dose of AII was increased at five-minute intervals until blood pressure was back at its baseline level. Details of the protocol have been given in 2.2.1.2.2.

The responses of blood pressure and renal hemodynamics to enalaprilic acid and AII have already been given in 4.1.2.

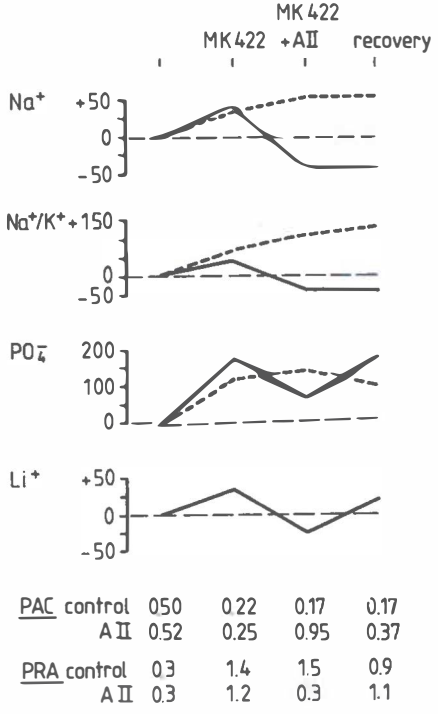


Figure 5.3:
Percentage change (median) in sodium excretion, sodium/potassium ratio, phosphate excretion and lithium clearance after enalaprilic acid (EA) alone (control day, broken lines) and after enalaprilic acid followed by AII (AII day, continuous lines). Median values of PRA and PAC are given at the bottom.

The effects of enalaprilic acid alone (control day) and of enalaprilic acid plus AII (AII day) on electrolyte excretion, PRA and PAC are given in *figure 5.3.* It shows that enalaprilic acid led to an increase in sodium excretion that was similar on the AII day and on the control day (only enalaprilic acid). Infusion of AII led to a distinct fall in sodium excretion to below baseline. Two hours after withdrawal of AII, sodium excretion was still suppressed, whereas on the control day it was still above baseline at the corresponding hours. The sodium/potassium ratio exhibited the same pattern. In the two patients in whom measurements were made up to four hours after AII, both sodium excretion and sodium/potassium ratio had returned to control by then. Phosphate excretion increased after enalaprilic acid but AII only par-

tially reversed this increase. After discontinuation of AII, phosphate excretion increased again. No measurements of lithium clearance were made on the control day. On the AII day lithium clearance increased after enalaprilic acid in all five patients. AII infusion led to a fall in this clearance to below baseline, after withdrawal of AII lithium clearance returned to baseline again.

On both the control day and the AII day PRA increased after enalaprilic acid. Infusion of AII led to a return to baseline whereas PRA increased again after discontinuation of AII. PAC fell after enalaprilic acid on both days; AII led to an increase of PAC to above baseline. Two hours after discontinuation of AII, PAC had fallen to below baseline again. At that time, however, PAC levels were still higher than the levels observed on the control day.

The responses of blood pressure and renal hemodynamics have been dealt with in detail in 4.1.2. Blood pressure decreased after enalaprilic acid in both experiments and could be titrated back to baseline by AII. ERPF increased after enalaprilic acid and GFR did not change. Both fell to below baseline after AII with a return to values not different from the control day after withdrawal of AII.

5.2 Enalapril.

5.2.1 Effects on sodium balance during low and liberal sodium intake.

In ten patients the effect of one week of treatment with enalapril on sodium balance was studied, both on a liberal (200 mmol Na^+/24h) and on a moderately restricted sodium intake(50 mmolNa^+/24h).

Table 5.II Electrolyte excretion, hormonal status and body weight. (n=10, mean ± SEM)

	baseline	day 1	day 7	sodium intake
UNaV (mmol/24h)	181±11**	202±19	188±12	200
	47±5	68±8	54±7	50
UKV (mmol/l)	73±5	66±5	74±6	200
	74±5	68±8	76±3	50
UcreatV (mmol/24h)	13.3±0.8	13.4±0.9	13.6±0.6	200
	13.7±1.0	13.4±0.8	13.4±0.7	50
PRA (nmolAI/l/h)	1.0±0.3*	3.2±1.0•	6.3±1.6•••	200
	2.3±0.8	5.1±1.2••	13.3±5.0•••	50
PAC (nmol/l)	0.42±0.07	0.26±0.06•••	0.39±0.08	200
	0.75±0.15	0.40±0.10•••	0.75±0.16	50
Body weight (kg)	76.1±3.2	75.6±3.1	75.2±2.4	200
	75.1±3.4	74.7±3.1	74.1±3.0••	50

Baseline values and the effects of enalapril on low liberal sodium intake.
*;**:p<0.02 and 0.01 respectively, paired test versus value on low sodium.
•; ••; •••: p< 0.05, 0.02 and 0.01 respectively, paired test versus baseline value.

Initial values for electrolyte excretion, PRA and PAC and body weight can be read from table 5.II, left column; the effects of enalapril on these variables from the middle and right columns. Sodium excretion increased at the institution of enalapril during both diets. In seven out of ten patients this increase was already apparent on the first day of therapy during both diets with a gradual return to baseline values during the next five days. Thereafter, excretion matched intake again. In three patients sodium excretion tended to decrease during the first two days of therapy, with subsequently a sharp increase on the next two to three days and a return to baseline thereafter. These three patients showed this pattern both on the restricted and the liberal sodium diet. As a result a net negative sodium balance could be calculated for all patients on both diets. Mean values are given in *figure 5.4*. On the restricted sodium diet a cumulated loss of 101 ± 24 mmol was found after six days of treatment; thereafter excretion matched intake again. On the liberal salt regimen the sodium loss was 112 ± 16 mmol; the difference between the regimens was statistically not significant. No consistent effects on potassium excretion were observed, consequently urinary sodium/potassium ratio increased on both sodium intakes.

Body weight fell with enalapril on both regimens; from 76.1 ± 3.2 to 75.2 ± 2.4 kg ($p<0.05$) on liberal sodium, and from 75.1 ± 3.4 to 74.1 ± 3.0 kg($p<0.02$) on restricted sodium.

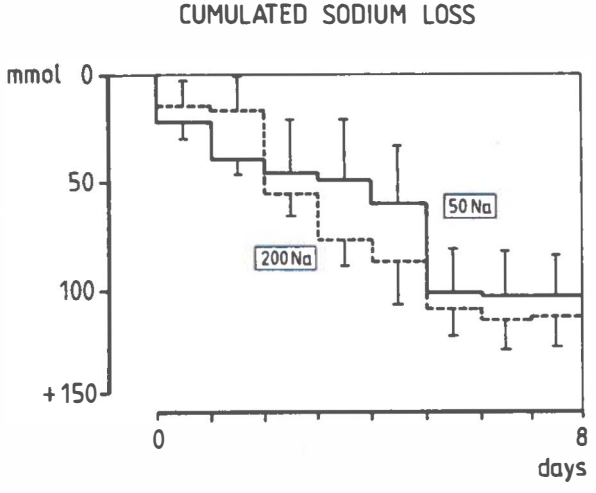


Figure 5.4: Effects of enalapril on cumulated sodium balance during liberal (broken lines) and during low (continuous lines) sodium diet. Mean ± SEM.

A sustained rise in PRA was observed after enalapril on either diet. PAC decreased in all patients at the onset of treatment, with a gradual return to baseline values during the next three to six days on either diet *(figure 5.5)*.

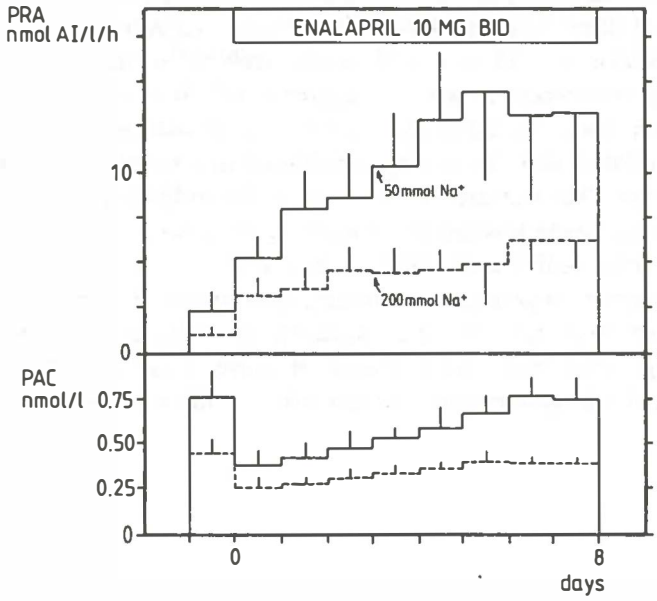


Figure 5.5: Effects of enalapril on PRA and PAC (mean ± SEM) on liberal (broken lines) and low (continuous lines) sodium diet.

Table 5.III Effects on serum electrolytes, uric acid and creatinine. (n=10, mean ± SEM)

	liberal sodium		low sodium	
serum	baseline	enalapril	baseline	enalapril
Na (mmol/l)	142±1	140±1	141±1	140±1
K (mmol/l)	4.3±0.1	4.5±0.2	4.4±0.1	4.5±0.2
uric acid (mmol/l	0.33±0.01	0.31±0.02	0.35±0.02	0.32±0.01*
creatinine (umol/l)	85±4	86±3	83±3	83±4

*; $p<0.01$, paired test versus baseline value.

The effects on serum electrolytes, creatinine and uric acid are given in Table 5.III. Uric acid decreased after enalapril on both sodium intakes. The changes in serum sodium and potassium did not reach statistical significance. The change in sodium excretion at institution of enalapril was

significantly and negatively correlated with the blood pressure response (r=-0.68; p<0.05) for both sodium intakes. Thus, the patients with the greatest fall in blood pressure had the smaller increase in sodium excretion or initially even retained some sodium. No significant correlation was found between the log of initial PRA and the responses of blood pressure and sodium excretion (day 1) either on the low (r=0.16 and -0.33, respectively) or on the liberal sodium intake (r=0.34 and -0.53, respectively).The changes in ERPF and GFR were not correlated with the log of initial PRA on either diet. None of the responses was related to age. The change in sodium excretion was positively correlated with the change in phosphate excretion (r=0.66 and 0.62, respectively). The natriuretic responses of the individual patients were virtually similar on the low and ond on the liberal sodium intake despite a considerable inter-individual variation, as depicted in *figure 5.6.*

The observation period was extended up to seven days after withdrawal of enalapril in eight patients. The results are given in *figure 5.7.* It shows, that seven days after withdrawal blood pressure, body weight and sodium balance had returned to values statistically not different from baseline on the

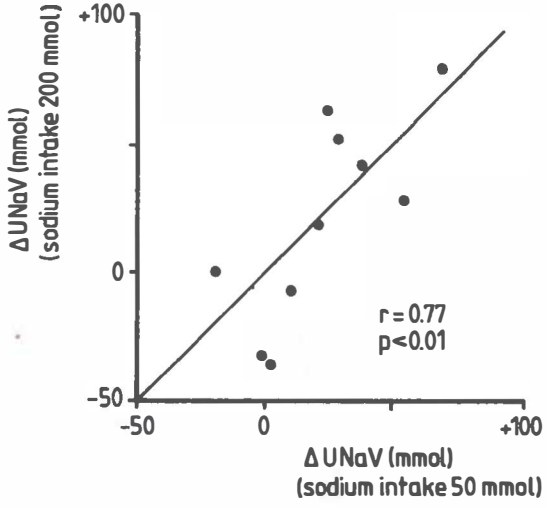


Figure 5.6: The change in urinary sodium excretion after enalapril during liberal (Y-axis) as compared to low sodium (X-axis) diet.

liberal sodium intake. On the low sodium diet the return to baseline was not complete. In one patient, a 52 year old man, the study periods on low and on liberal salt diet were, in this sequence, performed during the same hospitalization period allowing a strict comparison of the two. His data, given in *figure 5.8* show a more rapid return to baseline values after withdrawal of enalapril on liberal sodium intake.

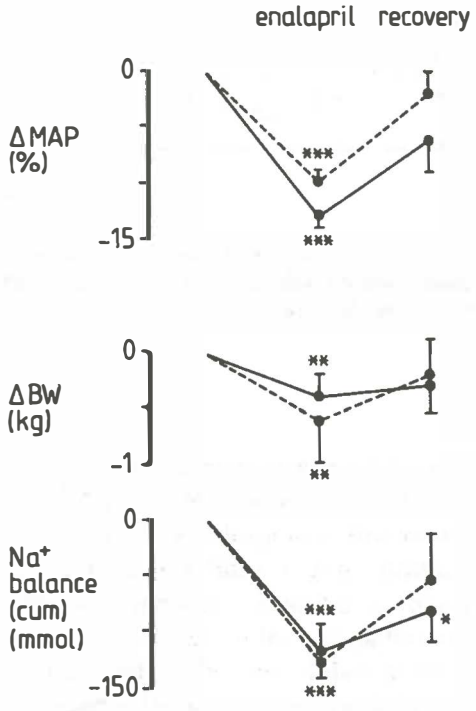


*Figure 5.7: Effect of institution and subsequent withdrawal of enalapril on MAP, body weight and cumulated sodium balance during low (continuous lines) and during liberal sodium intake (broken lines). Data are given as mean ± SEM, n=8. *; **; ***: p< 0.05; 0.02 and 0.01, respectively, paired test versus baseline value.*

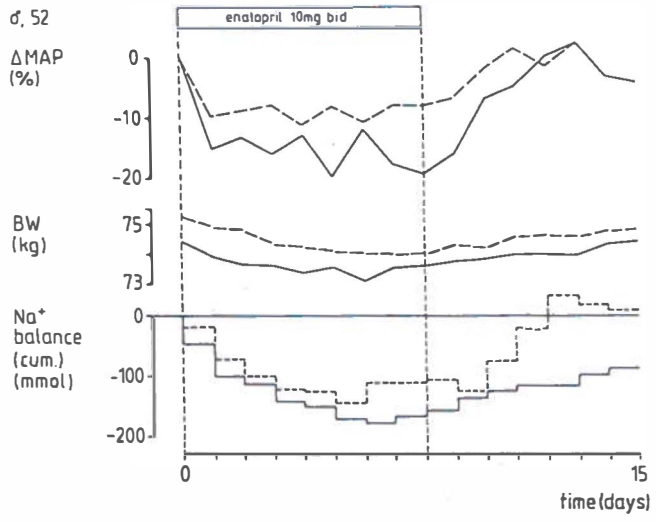


Figure 5.8: Effects of institution and subsequent withdrawal of enalapril on MAP, body weight and cumulated sodium balance in one individual on liberal (broken lines) and on low sodium diet (continuous lines).

Discussion.

The present chapter describes the effects of enalaprilic acid and enalapril on urinary sodium excretion. The studies were performed first, to establish whether acute ACE-inhibition with enalaprilic acid can promote sodium excretion, second, whether continued ACE-inhibition with enalapril induces a net sodium loss from the body, and finally to investigate whether the effect of enalapril depends on the initial state of sodium balance.

We found an acute increase in sodium excretion after enalaprilic acid as well as enalapril. Continued treatment with enalapril induced a net sodium loss, both on a low and a liberal sodium intake. The sodium loss was reversible after withdrawal of enalapril.

What could be the mechanism of the natriuresis? After enalaprilic acid as well as enalapril during liberal sodium intake, the natriuresis occurred without changes in GFR or creatinine excretion. Thus, an increase in GFR is

apparently not a decisive factor in the natriuresis. GFR, measured after one week enalapril on low sodium, had increased. This increase may have contributed to the natriuresis observed in this situation. This would imply a differently mediated natriuresis on low as compared to liberal sodium intake. The net effect on sodium excretion, however, was similar on both sodium intakes.

To assess whether altered tubular reabsorption contributes to the diuretic effect of enalaprilic acid and enalapril, we related the effects of enalaprilic acid and enalapril on sodium excretion to their effects on known markers of tubular sodium excretion. The change in phosphate excretion, a marker of proximal tubular sodium reabsorption (Seldin 1973), was positively correlated with the change in sodium excretion after enalaprilic acid as well as enalapril. This could imply that decreased tubular sodium reabsorption contributes to the natriuresis. In the studies on the effects of enalaprilic acid however, phosphate excretion not only increased after enalaprilic acid, but also after injection of placebo. This is in accord with the known diurnal excretion pattern of phosphate (Wesson 1964) and can be considered to confound the validity of phosphate excretion as a proximal marker in this setting. This restriction taken in mind, the correlation of the percentage increase in phosphate excretion with the log of initial PRA after enalaprilic acid (not found after placebo) as well as the only partial return to baseline of phosphate excretion after AII, do suggest a pharmacologically induced effect on phosphate excretion superimposed on the normal diurnal rhythm.

Lithium clearance, another marker of proximal tubular sodium reabsorption, increased in all five patients in whom it was measured. This again suggests that decreased proximal sodium reabsorption may contribute to the natriuresis. Urate excretion is known to be closely linked to proximal sodium handling and indeed the two may be coupled (Chonko, 1981). We did not measure the urinary excretion of urate. The decrease in serum uric acid after enalapril, however, again is consistent with decreased proximal sodium reabsorption.

How could this be mediated? The decrease in filtration fraction, after enalaprilic acid as well as enalapril, with the concomitant changes in peritubular hydrostatic and oncotic forces, can be expected to lead to a decrease in proximal sodium reabsorption. Alternatively, the abolition of a direct effect of AII on the proximal tubule could be involved (Harris 1984). Finally, a direct effect of either enalapril or enalaprilic acid, as has been suggested recently, could be involved (McNabb 1985).

An increase in the urinary sodium/potassium ratio, a marker of distal tubular sodium reabsorption was found after enalaprilic acid as well as enalapril. This implies that decreased distal sodium reabsorption also contributed to the natriuresis, probably due to the decrease in PAC.

We found a positive correlation between the log of initial PRA and the subsequent natriuretic response to enalaprilic acid, i.e. the patients with the highest initial PRA had the most pronounced natriuretic response. This is remarkable, as the patients with the highest initial PRA also had the most pronounced fall in blood pressure, which can be expected to counteract the increase in natriuresis (Guyton 1974, Omvik 1980). To further explore the role of decreased formation of AII in the natriuresis induced by enalaprilic acid we examined whether the renal effects of enalaprilic acid could be reversed by the infusion of exogenous AII. As shown in 4.1.2 indeed the renal hemodynamics effects of enalaprilic acid were abolished by AII. Sodium excretion, sodium/potassium ratio and lithium clearance (all increased after enalaprilic acid) fell to values below baseline after AII *(figure 5.3)*. PRA decreased and PAC increased after AII. Phosphate excretion was the only parameter that just partially returned to baseline after AII, probably due to its diurnal excretion pattern. Two hours after discontinuing AII, blood pressure, ERPF, GFR, the excretion of lithium and phosphate and PRA had returned to the values observed on the control day. Sodium excretion and sodium/potassium ratio were still below control. This could well be due to the incomplete return to control of PAC at that time, which is in accord with the half-life of circulating aldosterone (Morris 1981). Thus, infusion of exogenous AII reversed the effects on electrolyte excretion of enalaprilic acid both at the proximal and at the distal tubular level. Therefore, these are probably mediated by the decreased formation of AII, but our study does not rule out a role of other hormonal systems like the prostaglandin- and the kallikrein-kinin system.

Continued treatment with enalapril induced a net sodium loss from the body of about 100-120 mmol, on either sodium intake. This amount of sodium is equivalent to approximately 0.75 liter of extracellular fluid which is in accord with the weight loss we observed. After withdrawal of enalapril both sodium balance and body weight tended to return to baseline values. Within the timespan studied, the return was incomplete on the low sodium intake. This may reflect a more sluggish recovery on the low sodium intake, a phenomenon also encountered after withdrawal of diuretic therapy. Alternatively, it may indicate that we overestimated the sodium loss in our patients.

The natriuretic response to enalapril was similar on low and on liberal sodium. This is in some contrast with the responses of both blood pressure and renal hemodynamics, as these were both potentiated by low sodium diet. Why should the natriuretic response behave differently from the blood pressure response and the renal hemodynamic response in this respect? Several explanations are possible. First, the more pronounced fall in blood pressure

on the low sodium diet may have offset a more pronounced natriuresis. Second, it is possible that enalapril induced natriuresis not only by interference with the RAAS but also via other mechanisms, either through other hormonal systems or through a direct tubular effect. The latter can hardly be expected to be influenced by sodium status and the concomitant state of activation of the RAAS. Finally, the fact that the natriuretic responses in one individual so closely relate to each other may reflect some individual characteristic as to sodium handling that is of relevance to the responsiveness to ACE-inhibition.

We observed a sustained increase in PRA after enalapril on both diets. In contrast, PAC decreased only transiently with a return to baseline afterwards. How can this dissociation and in particular the return to baseline of PAC, be explained? First, it could be due to stimulation by AII. This would imply that still some AII is generated despite ACE-inhibition, for instance due to high levels of AI. We did not measure AII levels, but in studies by other investigators no evidence of such an escape phenomenon was found (Nüssberger 1985). The observation in our patients that the difference between both PRA and PAC on low as compared to liberal sodium intake persisted during enalapril treatment suggests that the axis sodium status-renin-angiotensin-aldosterone is still to some degree functioning under ACE-inhibition. If that be so, the sodium loss after enalapril could well contribute to the return to baseline of PAC. Second, the increase in aldosterone could be due to stimuli other than AII, e.g. ACTH and potassium. Especially subtle changes in potassium balance could play a role.

In spite of a decrease in potassium excretion after enalaprilic acid we found no consistent effects on potassium excretion at the institution of enalapril, in particular no potassium retention. Such a seeming discrepancy has been reported after captopril too (Brunner 1979). This might reflect the other part of the feed back loop between potassium and aldosterone, e.g. the return to baseline of PAC prevents potassium retention. Another explanation of the lack of potassium retention is found in the decrease in proximal sodium absorption, as an increased delivery of sodium to the distal tubulus is known to promote potassium excretion (Good 1979).

We conclude that enalaprilic acid promotes sodium excretion, despite a fall in blood pressure. Continued treatment with enalapril induces a net sodium loss from the body both on a low and on a liberal sodium intake. This diuretic effect may contribute to its antihypertensive action.

CHAPTER 6. GENERAL DISCUSSION.

In the previous chapters the effects of enalaprilic acid and enalapril on blood pressure, renal hemodynamics and sodium excretion have been described separately. We found that both enalaprilic acid and enalapril lower blood pressure, that both exert an effect on renal hemodynamics, and that both promote sodium excretion. These effects were studied with reference to two main questions. First, do the renal effects contribute to the antihypertensive effects and furthermore, are the renal effects of enalaprilic acid c.q enalapril specific for interference with the RAAS? In this general discussion the evidence gathered in the separate chapters will be reviewed and to some extent re-arranged to deal with these two questions.

The studies have focussed on three shifts of time. First, the acute effects as studied after injection of enalaprilic acid. Second, the short term effects, as studied at the institution of enalapril, and finally, the long term effects of maintenance treatment enalapril.

It has already been mentioned that net effects of antihypertensive treatment are the resultant of the pharmacological effects and the homeostatic responses of the organism, both of which are not constant in time. In general, the acute effects of an antihypertensive are considered to allow the more precise estimation of the pharmacological effects of the drug, whereas on maintenance treatment the net result is determined to a greater extent by the homeostatic responses and other non-drug-specific effects (Struyker-Boudier 1980). As such, the study of the acute effects of enalaprilic acid can be considered useful to unravel the pharmacological effects of ACE-inhibition. The studies of the effects of institution and maintenance treatment with enalapril on the other hand, allow an estimation of the eventual effects of ACE-inhibition in a more or less stationary condition after stabilization of the homeostatic responses.

As enalaprilic acid is the active metabolite of enalapril, its pharmacological effects are relevant to the action of enalapril. As to extrapolation of the

pharmacological effects of enalaprilic acid to those of enalapril caution is warranted, however, as the the two moieties have different pharmacokinetic properties. Animal studies have made it likely that enalapril, a less polar molecule than enalaprilic acid, enters various tissues more easily than enalaprilic acid (Cohen 1983, Unger 1982). Depending on the tissue involved, subsequent activation to enalaprilic acid occurs. Thus after administration of enalapril the concentration enalaprilic acid at tissue levels may be higher than after injection of enalaprilic acid. Therefore extrapolation of the acute effects of enalaprilic acid to the effects of enalapril is not justified.

In this chapter first the evidence concerning the specificity of the effects of enalaprilic acid and enalapril for interference with the RAAS is discussed. Then the mechanisms by which the kidney can lower blood pressure are briefly reviewed and finally the evidence linking the renal effects of enalapril to its antihypertensive effects will be discussed.

6.1 Specificity.

6.1.1 Enalaprilic acid.

As to the question whether the effects of enalaprilic acid, in particular the renal effects are specific for interference with the RAAS our data provide three lines of evidence. First, the correlation between the log of initial PRA and the acute effects of enalaprilic acid on blood pressure, ERPF and sodium excretion. Second, (only with respect to the effects on blood pressure) the effects of preceding renin stimulation by furosemide and altered sodium intake respectively. Third, the abolition of the effects of enalaprilic acid by the intravenous infusion of exogenous AII.

The correlation between the log of initial PRA and the effects of enalaprilic acid on blood pressure, ERPF and sodium excretion implies that these responses are more pronounced in patients with a high initial PRA. First it should be pointed out that a correlation, whatever its strength or statistical significance, cannot establish a causal relationship. This restriction taken in mind, the correlations we found are consistent with the notion that interference with a more activated RAAS exerts a more pronounced response. As such they point to interference with the RAAS as a mediator of the responses of blood pressure, ERPF and sodium excretion. They do not allow however, to ascribe the effects of enalaprilic acid exclusively to interference with the RAAS, and they do not allow to distinguish between the effects of a decrease in circulating AII and decreases in tissue (vascular wall, kidney, brain) AII.

Furthermore, it should be noted that these correlations were found within groups of patients on a standardized sodium intake, i.e. on a 50 mmol, a 100 mmol and on a 200 mmol sodium intake. So the initial PRA in each patient was the PRA for that given sodium intake. Strictly spoken, the difference in PRA levels under standardized sodium conditions reflects an individual characteristic as to the sodium and renin status. The individual susceptibility to the effects of ACE inhibition may be related to such an underlying characteristic rather than to the PRA level per se. For instance, a patient may have low PRA and not, or only slightly, increase his sodium excretion after ACE inhibition, both phenomenons due to an impaired capacity to excrete sodium. The considerable inter-individual variability in response for a given PRA level (or put otherwise, the relative weakness of the correlation between the log of initial PRA and the response to enalaprilic acid) moreover could imply that individual characteristics not reflected by the level of PRA contribute to the responsiveness to enalaprilic acid.

Renin stimulation by restriction of dietary sodium intake in one and the same patient leads to an increase, albeit small, of the blood pressure response. The potentiation is small when compared to the inter-individual variability. Nevertheless it demonstrates that individual susceptibility to ACE-inhibition, if any, is not a fixed characteristic, but subject to modification by the state of activation of the RAAS.

Renin-stimulation by pre-treatment with furosemide did not lead to a potentiation of the blood pressure response to enalaprilic acid. Moreover, the correlation between initial PRA and the blood pressure response to enalaprilic acid was lost after injection of furosemide. As discussed in chapter 3 this may be due to the pharmacological actions of furosemide other than induction of sodium loss. Within the context of the present discussion this finding demonstrates that the level of PRA does not directly reflect renin dependency of blood pressure, or the susceptibility of blood pressure for intervention by ACE inhibition.

We abolished the systemic and renal effects of enalaprilic acid by a graded intravenous infusion of exogenous AII. This could imply that the systemic and renal effects of enalaprilic acid are mediated by a decrease in the generation of AII.

As to the interpretation of these data several restrictions should be made. First, it could be argued with reason that AII would have led to an increase in blood pressure and a fall in ERPF, GFR and sodium excretion also if no enalaprilic acid had been given. Thus, the evidence derived from this study cannot be conclusive.

Second, we found a quantitative discrepancy in the responses to AII.

Doses sufficient to restore blood pressure to baseline led to a fall in ERPF, GFR and sodium excretion to values distinctly below baseline. The renal vascular bed is known to be much more sensitive to the vasoconstrictor action of circulating AII than the systemic vascular bed (Aurell 1969). This greater sensitivity explains why the renal response to exogenous AII was more pronounced than the blood pressure response. It does not explain, however, why the decrease in ERPF, GFR and sodium excretion after AII by far exceeded the increase in these parameters after enalaprilic acid, at least not if one assumes that the latter are solely due to a decrease in circulating AII levels. If that were true, one would expect exogenous AII to restore both blood pressure and renal function to baseline values in a quantitatively similar fashion. If, on the other hand, enalaprilic acid lowers blood pressure not only by a decrease in circulating AII levels, but also by a decrease in levels of AII on sites where the octapeptide AII has no access after intravenous administration (or alternatively by additional mechanisms not related to interference with the RAAS), higher doses of exogenous AII would be required to restore blood pressure to baseline, with consequently a sharp fall in ERPF, GFR and sodium excretion.

When making this inferences it should be kept in mind that ERPF was measured as the clearance of ^{131}I-Hippuran. A valid comparison of the effects of enalaprilic acid with those of AII would require that the renal extraction of Hippuran is not affected by either enalaprilic acid or AII. As to the effects of enalaprilic acid on renal extraction no data are available, but captopril is known to lower the extraction of Hippuran thus leading to an understimation of ERPF (Wenting 1984). Furthermore, AII also is known to affect extraction (Velasquez 1972). Therefore, our results need confirmation either by a similar study including extraction measurements, or by a study assessing renal blood flow by a method not dependent on renal extraction.

6.1.2 Enalapril.

With enalapril no significant correlation could be found between the log of initial PRA and the effects on blood pressure, renal hemodynamics and sodium excretion. This was true for the long term effects (on a sodium intake of 100 mmol) as well as the effects after one week of treatment and the first-dose effect on a sodium intake of either 50 or 200 mmol/24h.

This is in seeming contrast with our findings after enalaprilic acid as well as the experience with captopril and teprotide. Does this mean that enalapril does not exert its effects by interference with the RAAS? It has already been pointed out that the pharmacological actions of a drug are most readily assessed after acute administration, as on prolonged administration homeostatic

reactions tend to obscure the pharmacological effects. After captopril for instance, the correlation between the initial blood pressure response and initial PRA gradually gets weaker during the first weak of treatment and eventually disappears. Therefore the absence of such a correlation on maintenance treatment enalapril could be anticipated. The absence of such a correlation for the blood pressure response to the first dose of enalapril deserves more consideration, as it contrasts with the findings after the ACE-inhibitors captopril, teprotide and enalaprilic acid. This may reflect the fact that of these ACE-inhibitors enalapril has the slowest onset of action. Alternatively, it may indicate that the circulating renin level is less relevant to the actions of enalapril than to the action of the named ACE-inhibitors. It could be that the less polar molecule enalapril derives a greater part of its acute actions from activity at the tissue level.

Finally the absence of a correlation between initial PRA and the first-dose effect of enalapril could reflect a statistical phenomenon rather than a biological one as the first-dose effect was studied in only ten patients.

Sodium restriction with the concomitant increase in the activity of the RAAS led to a potentiation of the hemodynamic responses to enalapril, of the systemic as well as the renal vascular bed. This is consistent with the notion that interference with the RAAS is an important mode of action of enalapril.

The potentiation of the blood pressure response, however, was unimpressive, especially when compared to the inter-individual variability, both for a given sodium intake and for a given PRA level. Just like the findings with enalaprilic acid, these data suggest an individual susceptibility to the actions of enalapril that, albeit not exclusively determined by the prevalent RAAS-activity, is subject to modification by the state of activation of the RAAS.

6.2 Do the renal effects contribute to the antihypertensive effects?

6.2.1 Renal antihypertensive mechanisms.

The central role of the kidney in blood pressure regulation has already been emphasized in paragraph 1.3. The mechanisms by which the kidney can lower blood pressure can be grossly divided into two categories. First, those linked to the function of the kidney as an excretory organ, and second, those linked to the function of the kidney as an endocrine organ. In vivo these two operate in close interaction.

In brief the kidney can exert its effects on blood pressure by the regulation of sodium and volume status on one hand, and by the release of vaso-active

substances on the other hand. The latter include the vasopressor cascade of the renin-angiotensin system, as well as a less well defined vasodepressor system, probably residing in the renal medulla (Muirhead 1980). The studies described in this thesis mainly concentrate on the first category, i.e. on the effects of increased sodium excretion as a possible mechanism of the antihypertensive action of enalapril. Therefore the relevance of increased sodium excretion to antihypertensive drug action will be discussed in some more detail.

It has long been known that drugs inducing increased sodium excretion can lead to a fall in blood pressure. Indeed large scale antihypertensive treatment was first made possible by the introduction of the thiazide diuretics (Dustan 1974, 1983, Zanchetti 1985). It has been a matter of dispute whether their antihypertensive effect was directly linked to the induced sodium loss. As to the acute effects of thiazides as well as furosemide a direct link between sodium loss and antihypertensive effect has been elegantly shown by the experiments of Finnerty and Davidow (Davidow 1969, Finnerty 1968). They demonstrated that these diuretics did not lower blood pressure when a decrease in extracellular fluid volume was prevented by an exact replacement of the loss of sodium and fluids by intravenous infusion of saline- or by a much larger infusion of glucose 5%.

On maintenance treatment however, the relationship between sodium loss, decreased extracellular fluid volume and antihypertensive effect is more complicated. Indeed an inverse correlation between reduction in extracellular fluid volume and antihypertensive effect has been demonstrated (van Brummelen 1980). Thus, a persistent reduction in extracellular fluid volume on maintenance treatment is associated with a poor blood pressure response, whereas in patients with a good blood pressure response on maintenance treatment the extracellular fluid volume eventually returns to baseline. One of the explanations of the poor blood pressure response in patients with a persistent decrease in extracellular fluid volume is excess activation of the RAAS, with consequently AII-mediated vasoconstriction.

Does this mean that a diuretic effect, or a reduction of body sodium cannot play a role in a long term reduction of blood pressure? This would be in striking contrast with the statements made in paragraph 1.3, namely, that the principal mechanism of long term blood pressure control is the relationship between arterial pressure and sodium excretion (Borst 1963, Guyton 1974)! This notion has found confirmation from several lines of evidence, derived from the field of experimental hypertension as well as from the clinical experience with antihypertensive drugs. As to antihypertensive therapy, it has been shown for several types of drugs that their long term efficacy is related

to the presence or the absence of secondary sodium retention (Dustan 1983). Indeed this notion has for two decades formed the rationale of the combination of vasodilators and sympatholytic agents with diuretics (Gifford 1984, Zanchetti 1985).

The seeming contradiction is solved when one considers the time course in antihypertensive treatment. Blood pressure can acutely be lowered by a variety of mechanisms, i.e. vasodilation leading to a fall in total peripheral resistance, a negative inotropic effect leading to a fall in cardiac output, and an increased sodium excretion, leading to a decrease in extracellular fluid volume and consequently cardiac output. Every decrease in blood pressure elicits a series of homeostatic responses. If the drug is rapidly acting and only a single dose is administered these will mainly be short term mechanisms, i.e. nervous reflexes. On maintenance therapy homeostatic mechanisms that require several days to become fully operative come into play. The relationship between arterial pressure and sodium excretion i.e. long term volume control is the most important of these.

Thus, pharmacological effects on sodium balance can be relevant to the lowering of blood pressure in two ways. First, increased sodium excretion can directly lower blood pressure in the acute situation by a decrease in extracellular fluid volume and consequently cardiac output. Second, when blood pressure is initially lowered by another mechanism, e.g. a decrease in total peripheral resistance or a decrease in cardiac output, the effects of the drug on sodium excretion become relevant in second instance, as it is the state of sodium balance that determines long term antihypertensive efficacy to an important extent.

6.2.2 Enalaprilic acid, enalapril

What evidence do our studies provide linking the natriuretic effects of enalaprilic acid and enalapril to their effects on blood pressure, one way or another?

Enalaprilic acid and enalapril both acutely increase sodium excretion in the majority of the patients, on either sodium intake. Enalaprilic acid lowered blood pressure within 11 minutes in all patients. Sodium excretion increased within an hour after injection in 11 out of 14 patients. The mean excess sodium excretion after one hour was 1 ± 1mmol/hr(ns). It is hardly probable that this minute effect has contributed to the fall in blood pressure. Most likely the initial fall in blood pressure is due to vasodilation secondary to decreased levels of AII.

If the increase in sodium excretion would contribute to the fall in blood pressure one would expect that patients with the most pronounced increase

in natriuresis or the greatest overall sodium loss also had the most pronounced blood pressure response- as has been demonstrated for the acute effects of hydrochlorothiazide (Davidow 1969, Finnerty 1968). No such relationship could be established for the blood pressure response to enalaprilic acid in our patients. No significant correlation could be found between either the acute increase in sodium excretion or the total amount of sodium excreted in excess to baseline, and the fall in blood pressure. Therefore, from our data it is not likely that the hypotensive effect of enalaprilic acid is mediated wholly, or in part by an increase in sodium excretion and the subsequent fall in extracellular fluid volume.

After enalapril the excess sodium excretion on the first day amounted to 79 mmol, equivalent to 0.5 liter of extracellular fluid. The patients with the most pronounced acute fall in blood pressure had the smallest initial increase in sodium excretion or even retained some sodium. This is highly suggestive of an antinatriuretic effect of the fall in blood pressure and obviously does not suggest that the fall in blood pressure is due to an increased sodium excretion in a "thiazide-like" mechanism. Most probably the acute fall in blood pressure after enalapril is due to vasodilation secondary to decreased AII levels.

Yet, as pointed out in 6.2.1 the increased natriuresis could still contribute to the fall in blood pressure in a less direct way. If one assumes that vasodilation is the primary mechanism of action of enalapril, accounting for the initial fall in blood pressure, a negative sodium balance over the first days of treatment will further lower blood pressure in the following days. Therefore we related the fall in blood pressure between the first and the eighth day of treatment with the increase in sodium excretion *(figure 6.1)*. Indeed we found that the patients that showed the most pronounced sodium excretion at the onset of treatment (day one) had a more pronounced fall in blood pressure during the subsequent days on either sodium intake. This is consistent with a hypotensive effect of the sodium loss superimposed on the hypotensive effects of vasodilation.

It should be emphasized however, that this provides only indirect evidence and no conclusive proof. Strictly logical the data on the inverse relationship between the fall in blood ressure and the natriuresis on the first day of treatment, combined with the apparently reversed relationship during the following days, only mean that in patients with a gradual onset of the antihypertensive effect the initial natriuresis is more pronounced. As such these relationships illustrate that the net effects of enalapril on sodium excretion are determined by two opposite forces. On one side the antinatriuretic effects of the fall in systemic arterial pressure, mediated by vasodilation. On the

other side the natriuretic renal effects, due to the tubular effects of decreased levels of AII and aldosterone and the changes in renal hemodynamics. The latter could contribute to the hypotensive effect. This delicate balance is demonstrated in the individual data of two patients in *figure 6.2.*

The patient depicted in the left panel has a reaction to enalapril that is characterized by a gradual fall in blood pressure and an immediate natriuresis on both diets. It is conceivable that with this type of response the sodium loss during the first few days of treatment contributes to the secondary fall in blood pressure. In the patient depicted in the right panel however, the response follows a different pattern; a rapid blood pressure reduction with a partial return to baseline after a few days treatment. In this patient, after an initial decrease, sodium excretion rises sharply once the fall in blood pressure has passed its nadir, and only then sodium balance becomes negative. From these observations it does not seem very likely that within the timespan studied the natriuresis contributed to the fall in blood pressure in this patient. Whatever the mechanism, it is an intriguing observation, that some patients respond to ACE-inhibition primarily with a fall in blood pressure, whereas others respond primarily with natriuresis.

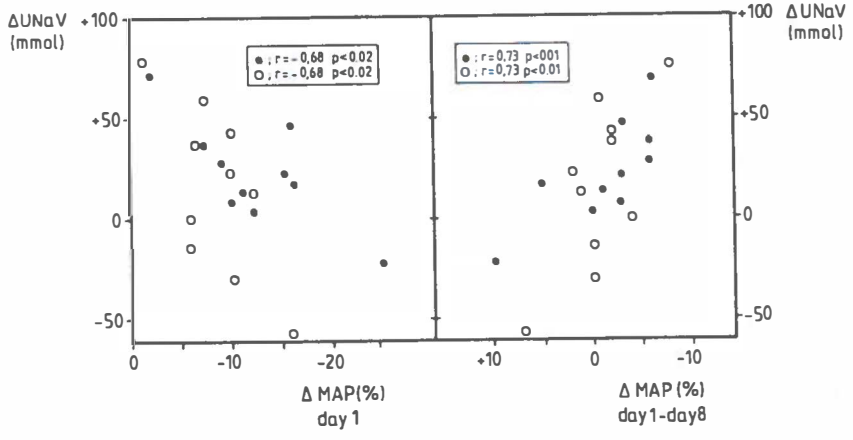


Figure 6.1: The correlation of the increase in natriuresis after enalapril on day 1 with the fall in blood pressure on the first day of treatment (day 1, left panel) and with the fall in blood pressure between the first and eight day of treatment (day 1-day 8, right panel). Data on 50 mmol sodium diet are represented by closed circles, data on 200 mmol sodium diet by open circles.

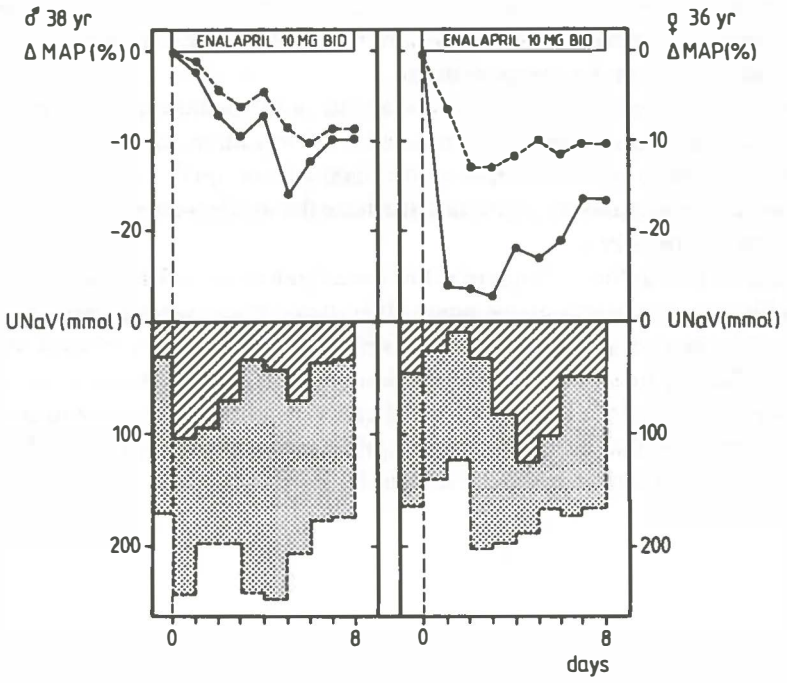


Figure 6.2: Individual data of two patients showing the fall in MAP (upper panel) on 50 mmol sodium (continuous lines) and on 200 mmol sodium (broken lines), and the daily sodium excretion (lower panel) on both diets.

6.3 Conclusions.

The studies described in this thesis concentrated on the effects of enalaprilic acid and enalapril on blood pressure, renal hemodynamics and sodium excretion. We found that both enalaprilic acid and enalapril are effective in the lowering of blood pressure. The place of enalaprilic acid in antihypertensive therapy will probably remain limited and has to be defined by further study. Enalapril is a suitable drug for the management of essential hypertension and it can succesfully be used as a first line of therapy. Both enalaprilic acid and enalapril induce renal vasodilation and natriuresis. Our special interest was to establish, first, whether the renal effects contribute to the antihypertensive effects and furthermore, whether these effects are specific for interference with the RAAS.

Neither after enalapril, nor after enalaprilic acid we found effects that could not be explained by interference with the RAAS. Furthermore the sus

ceptibility to the effects of enalapril as well as enalaprilic acid was modified by the state of activation of the RAAS. Therefore, our results are consistent with interference with the RAAS as an important mode of action; yet they do not exclude a role for other systems.

Not surprisingly the effects of enalaprilic acid resembled those of enalapril; it lowered blood pressure, it induced renal vasodilation and it induced an increased natriuresis. Only the acute effects of enalaprilic acid were studied, within this time span we could not attribute the antihypertensive effect to the increased natriuresis.

In accord with the findings of other investigators we found that enalapril is an effective antihypertensive agent. It induces renal vasodilation. In addition, it induces a net sodium loss both on a low and on a liberal sodium intake. Taking into account the time course of the antihypertensive action of enalapril, we could demonstrate a delicate balance between natriuresis and blood pressure decrease. Thus, enalapril derives its efficacy from the combination of a vasodilatory and a diuretic effect.

SUMMARY.

The studies described in this thesis deal with the effects of the orally active angiotensin converting enzyme (ACE)-inhibitor enalapril and its active metabolite enalaprilic acid in the treatment of essential hypertension, with special emphasis on its effects on renal hemodynamics and sodium excretion.

ACE-inhibitors were specifically developed to interfere with the renin-angiotensin-aldosterone system (RAAS), as the RAAS had since long been suspected to play a role in the pathogenesis of hypertension. ACE-inhibitors indeed effectively lower blood pressure, and they have rapidly been introduced in the treatment of hypertension. The mechanism by which ACE-inhibitors lower blood pressure is not quite clear. As ACE-inhibitors have distinct renal effects, and as the kidney has a central role in volume homeostasis and the long-term regulation of blood pressure, it might well be that the renal effects of ACE-inhibitors contribute to their hypotensive action. The present study therefore focusses on the effects of enalapril on blood pressure, renal hemodynamics and sodium excretion to assess whether the renal effects contribute to the effects on blood pressure. Furthermore, the question whether the effects of enalapril are specific for interference with the RAAS is addressed indirectly, mainly by investigating the influence of the pre-existent state of activation of the RAAS on the effects of ACE-inhibition.

Chapter two describes the study protocols and the methods used. The effects of enalapril and enalaprilic acid are described in chapter three. With enalaprilic acid we first performed a dose-finding study aimed at finding the optimal dose for rapid blood pressure reduction in patients on a sodium intake of 100 mmol Na^+/24h. Within the dose-range of 5 to 80 mg the dose-response appeared flat, whereas doses below 5 mg were less effective. We subsequently tried to augment the blood pressure response to a fixed dose of 10 mg enalaprilic acid by preceding treatment with furosemide. Despite effective natriuresis and despite renin-stimulation by furosemide no potentiation of the blood pressure response to enalaprilic acid was found. We then investigated whether the blood pressure response was influenced by sodium intake; it appeared that the response was slightly but consistently more pronounced on a moderately restricted (50 mmol Na^+/24h) than on a liberal (200 mmol Na^+/24h) sodium intake.

As to enalapril we compared its efficacy as a first line of treatment in outpatients with mild to moderate and moderate to severe essential hypertension respectively, with conventional stepped care. In our study the regimens with enalapril as first line drug and the conventional regimens with propranolol

and hydrochlorothiazide as first drug were equally effective. We then investigated whether moderate sodium restriction augments the blood pressure response to enalapril. Indeed the blood pressure response appeared to be slightly but consistently more pronounced on a 50 mmol sodium as compared to a 200 mmol sodium diet.

The effects of enalapril and enalaprilic acid on renal hemodynamics are given in chapter four. Enalaprilic acid acutely induces a rise in effective renal plasma flow (ERPF), despite a fall in blood pressure, without change in glomerular filtration rate (GFR). Consequently filtration fraction (FF) falls. A subsequent graded infusion of angiotensin II (AII), in doses sufficient to titrate blood pressure back to baseline results in sharp fall of ERPF and GFR to below baseline. Thus, the renal hemodynamic effects of enalaprilic acid could be annihilated by AII, but there was a quantitative discrepancy in the effects on blood pressure and renal hemodynamics.

The effects on renal function of maintenance treatment enalapril as first-line drug were compared with the effects of conventional regimens. During the first twelve weeks of treatment with enalapril we found an increase in ERPF, without change in GFR, and consequently a fall in FF. No such change was found with conventional treatment. On prolonged treatment however, the increase in ERPF with enalapril tended to disappear, whereas GFR fell somewhat with both the enalapril regimens and the control regimens. Consequently the remaining difference in renal hemodynamic response after one year of treatment was a fall in FF with enalapril. Whether this constitutes a benefit for the patient remains to be proven. Finally, we studied the effect of a moderate restriction of sodium intake on the renal hemodynamic response to one week of treatment with enalapril in a cross-over study. On a 50 mmol sodium intake the baseline value of GFR, and less consistently ERPF, was somewhat lower than on a 200 mmol sodium intake. On 50 mmol sodium enalapril resulted in rise of both ERPF and GFR, whereas on 200 mmol sodium the rise in ERPF after enalapril was less pronounced, and no change in GFR occurred. As a consequence, the difference in renal hemodynamics between restricted and liberal sodium was no longer apparent after enalapril. Most likely therefore the renal hemodynamic response to moderate sodium restriction is mediated by the RAAS.

Chapter five describes the effects of enalaprilic acid and enalapril on sodium excretion. Enalaprilic acid induces an acute increase in sodium excretion, accompanied by a fall in potassium excretion. This increased natriuresis is probably due to a decrease in both proximal and distal tubular sodium reabsorption. Institution of enalapril leads to a net sodium loss from the body during the first few days of treatment. Strikingly, the sodium loss is

similar on a moderately restricted as compared to a liberal sodium intake. This negative sodium balance is accompanied by a fall in body weight and it is reversible after withdrawal of nalapril.

Chapter six reviews the evidence for the specificity of the effects of enalapril for interference with the RAAS and finally the evidence linking the renal effects to the effects on blood pressure is discussed. It is argued that a delicate balance exists between the hypotensive and the natriuretic effects of enalapril, and that this balance expresses itself differently in different individuals. It is concluded that enalapril derives its efficacy from a combination of vasodilatory and diuretic effects.

SAMENVATTING

Dit proefschrift behandelt de effecten van de oraal werkzame angiotensine-converting enzyme (ACE)-remmer enalapril en zijn aktieve metaboliet enalaprilic acid op bloeddruk, nierfunktie en natriumuitscheiding bij de behandeling van essentiele hypertensie. De ontwikkeling van ACE-remmers als antihypertensiva is het resultaat van doelgericht onderzoek naar de mogelijkheid tot blokkade van het renine-angiotensine-aldosteron systeem (RAAS). Van dit systeem werd namelijk al lang verondersteld, dat het een rol speelt in de pathogenese van verhoogde bloeddruk.

ACE-remmers bleken inderdaad de bloeddruk te verlagen en zijn inmiddels geintroduceerd in de behandeling van hypertensie. Hoe de bloeddrukverlagende werking van ACE-remmers tot stand komt is niet volledig opgehelderd. Daar ACE-remmers uitgesproken effekten hebben op de nierfunktie, en daar de nier een centrale rol speelt in de volumehomeostase en de bloeddrukregulatie, zijn wellicht de renale effecten van ACE-remmers van belang voor de bloeddrukverlagende werking. In het hier beschreven onderzoek worden daarom de effecten van enalapril op bloeddruk, renale hemodynamiek en natriumuitscheiding onderzocht in hun samenhang, vanuit de vraagstelling of de renale effecten bijdragen tot de bloeddrukverlagende werking.

In hoofdstuk twee worden de protocollen en de gebruikte methoden beschreven. Hoofdstuk drie beschrijft de effecten van enalapril en enalaprilic acid op de bloeddruk. Met enalaprilic acid werd een dose-finding studie uitgevoerd, bij patienten op een dieet met 100 mmol Na^+/24h. Doel was het vaststellen van de juiste dosis voor een snelle bloeddrukreductie. Doses beneden de 5 mg bleken niet effectief in dit opzicht. In de range van 5 tot 80 mg was de dosis-response curve vlak. Vervolgens werd onderzocht of de bloeddrukreactie na 10 mg enalaprilic acid te versterken was door de patienten vóór te behandelen met een injectie furosemide. Furosemide stimuleerde zowel de natriurese als het plasma renine gehalte, maar dit leidde niet tot een versterkte bloeddrukdaling na enalaprilic acid. Vervolgens werd onderzocht of de bloeddrukreactie afhankelijk is van de hoeveelheid natrium in het dieet. Inderdaad bleek de bloeddrukreactie bij een dieet met 50 mmol Na^+/24h iets meer uitgesproken dan bij een natriuminname van 200 mmol/24h.

De effectiviteit van enalapril als middel van eerste keuze in patienten met lichte tot matige en matige tot ernstige hypertensie bleek in een poliklinisch onderzoek overeen te komen met de effectiviteit van twee conventionele stepped-care regimes met propranolol respectievelijk hydrochlorothiazide als middel van eerste keuze. Vervolgens werd de invloed van het natrium

gehalte van het dieet op de bloeddrukreactie op enalapril onderzocht; de bloeddrukdaling bleek iets sterker bij een natriuminname van 50 mmol dan bij een natriuminname van 200 mmol.

De effecten van enalapril en enalaprilic acid op de renale hemodynamiek worden beschreven in hoofdstuk vier. Enalaprilic acid leidt tot een stijging in effectieve renale plasma flow (ERPF) ondanks de bloeddrukdaling, zonder dat de glomerulaire filtratiesnelheid (GFR) verandert. Dientengevolge daalt de filtratie fractie (FF). Wordt na toediening van enalaprilic acid de bloeddruk door middel van een intraveneuze infusie met angiotensine II (AII) op het uitgangsniveau teruggebracht, dan treedt een scherpe daling op van ERPF en GFR tot beneden het uitgangsniveau. AII heft dus de renale effecten van enalaprilic acid op, maar er bestaat een kwantitatieve discrepantie tussen de effecten op de bloeddruk en die op de renale hemodynamiek.

De effecten van een onderhoudsbehandeling met enalapril op de renale hemodynamiek werden vergeleken met de effecten van conventionele therapie. Na twaalf weken behandeling werd met enalapril een stijging in ERPF gevonden zonder verandering in GFR, zodat de FF gedaald was. Deze verandering werd niet gevonden in de controlegroep. Na een jaar behandeling was de stijging in ERPF met enalapril nagenoeg verdwenen; tevens was zowel met enalapril als met conventionele therapie de GFR iets gedaald. Dientengevolge was het resterende verschil na een jaar behandeling een daling van de FF met enalapril. Of dit voor de patient van voordeel is, is op grond van onze gegevens niet uit te maken. Tenslotte werd in een cross-over experiment onderzocht of de natriuminname van invloed is op de renale respons op enalapril. Bij gebruik van 50 mmol Na^+/24u bleek in de onbehandelde patienten de GFR, en in mindere mate de ERPF, iets lager dan bij gebruik van 200 mmol Na^+/24u. Behandeling met enalapril leidde tot een stijging in ERPF zowel als GFR bij gebruik van 50 mmol Na^+, en tot een minder uitgesproken stijging van ERPF zonder effect op GFR bij gebruik van 200 mmol Na^+. Opvallend was, dat dit verschil in respons op enalapril juist zodanig was, dat daarmee de verschillen in uitgangswaarden van GFR en ERPF werden gecompenseerd. De lagere waarden voor GFR en ERPF bij gebruik van het natrium beperkte dieet komen dus waarschijnlijk tot stand door middel van RAAS-gemedieerde vasoconstrictie.

De effecten van enalaprilic acid en enalapril op de natrium uitscheiding worden behandeld in hoofdstuk vijf. Enalaprilic acid leidt tot een onmiddellijke toename in natrium uitscheiding, die gepaard gaat met een daling in kalium uitscheiding. Deze natriurese komt waarschijnlijk tot stand door een afname in zowel de proximale als de distale tubulaire natrium terugresorptie.

Behandeling met enalapril leidt tot een netto negatieve natrium balans gedurende de eerste drie tot vijf dagen behandeling. Opvallend is, dat het natriumverlies gelijk is bij gebruik van 50 mmol Na^+/24u en bij 200 mmol Na^+/24u in het dieet.

In hoofdstuk zes tenslotte worden de eerder gepresenteerde gegevens opnieuw gegroepeerd en besproken wordt in hoeverre de gevonden effecten op bloeddruk, renale hemodynamiek en natrium uitscheiding specifiek zijn voor blokkade van het RAAS, en in hoeverre het gevonden diuretisch effect zou kunnen bijdragen tot het bloeddrukverlagend effect. Uiteengezet wordt, dat een dynamisch evenwicht bestaat tussen het bloeddrukverlagend effect en het natriuretisch effect van enalapril. In verschillende individuen komt dit evenwicht op verschillende wijze tot expressie. Geconcludeerd wordt, dat het bloeddrukverlagend effect van enalapril tot stand komt door een combinatie van vaatverwijding en natriurese.

REFERENCES.

Ackerman DM, Weinstock J, Wiebelhaus VD, Berkowitz B (1982).
Renal vasodilators and hypertension. Drug Dev Res 2; 283-297.

Amery A, Birkenhäger WH, Brixco P et al (1985).
Mortality and morbidity results from the European Working Party on Hypertension in the Elderly Trial. Lancet i; 1349-1354.

Ames RP, Hill P (1982).
Improvement of glucose tolerance and lowering of glycohemoglobin and serum lipid concentrations after discontinuation of antihypertensive therapy. Circulation 65; 899-904.

Anderson TW (1978).
Re-examination of some of the Framingham data. Lancet ii; 1139-1141.

Antonaccio MJ, High JP, Rubin B, Schaeffer T (1979).
Contribution of the kidneys but not adrenal glands to the acute antihypertensive effects of captopril in spontaneously hypertensive rats. Clin Sci 57; 127s-130s.

Atkinson AB, Brown JJ, Lever AF, Robertson JIS (1980).
Combined treatment of severe intractable hypertension with captopril and diuretic. Lancet ii; 105-110.

Atlas SA, Case DB, Sealey JE, Laragh JH, McKinstry DN(1979).
Interruption of the renin-angiotensin system in hypertensive patients by captopril induces sustained reduction in aldosterone excretion, potassium retention and natriuresis. Hypertension 1; 274-280.

Aukland K (1976).
Renal blood flow. In: International review of physiology. Kidney and urinary tract physiology II, vol 11. K Thurau (Ed). University Park Press, Baltimore, pp 47-51.

Aurell M (1969).
Renal response in man to plasma volume expansion and angiotensin. Scand J Clin Lab Invest 24; supp 112.

Australian National Blood Pressure Study Management Committee (1980).
The Australian therapeutic trial in mild hypertension. Lancet i; 1261-1267.

Bacon PA, Tribe CR, Mackenzie JC, Verrier-Jones J, Cumming RH, Amer B (1976).
Penicillamine nephropathy in rheumatoid arthritis; a clinical pathological and immunological study. Quart J Med 45; 661-684.

Baylis C (1983).
Control of Na^+-reabsorption and its relation to renal hemodynamics. Seminars in nephrology 3; 180-194.

Bechgaard P, Kopp H, Neilson J (1956).
One thousand hypertensive patients followed from 16-22 years. Acta Med Scand 154, supp 312; 175-184.

Bengis RG, Coleman TG, Young DB, McCaa RE (1981).
Long-term blockade of angiotensin formation in various normotensive and hypertensive rat models using converting enzyme inhibition(SQ 14,225). Circ Res 43, supp I; 45-53.

Bergstrand R, Johansson S, Vedin A, Wilhelmsson C (1982).
Comparison of once-a-day and twice-a-day dosage regimens of Enalapril (MK 421) in patients with mild hypertension. Br J Clin Pharmacol 14; 136P-137P.

Biollaz J, Brunner HR, Gavras I, Waeber B, Gavras H (1982).
Antihypertensive therapy with MK 421; angiotensin II-renin relationships to evaluate efficacy of converting enzyme blockade. J Cardiovasc Pharmacol 4; 966-972.

Biollaz J, Schelling JL, Jacot des Combes B et al (1982).
Enalapril maleate and a lysine analogue (MK 521) in normal volunteers; relationship between plasma drug levels and the renin angiotensin system. Br J Clin Pharmacol 14; 363-368.

Blacket RB, Depoorter A, Pickering GW, Sellers AL, Wilson GM (1950).
Hypertension produced in the rabbit by long-continued infusions of renin. Clin Sci 9; 223-245.

Blantz RC, Konnen KS, Tucker BJ (1976).
Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. J Clin Invest 57; 419-443.

Bock KD, Krecke H-J (1958).
Die Wirkung von synthetischen Hypertensin II auf die PAH- und die Inulin-clearance, die renale Hämodynamik und die Diurese beim Menschen. Klin Wochenschr 36; 69-74.

Borst JGG, Borst-de Geus A (1963).
Hypertension explained by Starlings theory of homeostasis. Lancet i; 677-683.

Braun-Menendez E, Fasciolo JC, Leloir LF, Munoz JN (1940).
The substance causing renal hypertension. J Physiol 98; 283-298.

Bravo EL, Tarazi RC (1979).
Converting enzyme inhibition with an orally active compound in hypertensive man. Hypertension 1; 39-46.

Breckenridge A (1985).
Treating mild hypertension. Br Med J 291; 89-90.

de Bruyn JHB, Wenting GJ, Man in't Veld AJ, Derkx FHM, Schalekamp MADH (1980).
Captopril affects blood pressure equally in renovascular and essential hypertension and in the fluid-depleted anephric state.Clin Sci 59; 83s-86s.

de Bruyn JHB, Man in't Veld AJ, Wenting GJ, Derkx FHM, Schalekamp MADH (1981).
Haemodynamic profile of captopril in various forms of hypertension. Eur J Clin Pharmacol 20; 163-168.

van Brummelen P, Man in 't Veld AJ, Schalekamp MADH (1980).
Sequential hemodynamic changes during long-term thiazide treatment of essential hypertension in responders and non-responders. Clin Pharmacol Ther 27 ; 328-336.

Brunner HR, Gavras H, Waeber B et al (1979).
Oral angiotensin converting enzyme inhibitor in long term treatment of hypertensive patients. Ann Intern Med 90; 19-23.

Brunner HR, Gavras H, Waeber B, Textor SC, Turini GA, Wauters JP (1980).
Clinical use of an orally active converting enzyme inhibitor; captopril. Hypertension 2; 558-566.

Bumpus FM, Smeby RR, Page IH, Khairallah PA (1964).
Distribution and metabolic fate of angiotensin II and various derivatives. Can Med Assoc J 90; 190-193.

Campbell BC, Sturani A, Reid JL (1985).
Evidence of parasympathetic activity of the angiotensin converting enzyme inhibitor captopril in normotensive man. Clin Sci 68; 49-56.

Case DB, Wallace JM, Keim HJ, Weber MA, Sealey JE, Laragh JH (1977).
Possible role of renin in hypertension as suggested by renin-sodium profiling and inhibition of converting enzyme. N Engl J Med 296; 641-646.

Chasis H, Goldring W, Breed ES, Schreiner GE, Bolomey AA (1950).
Salt and protein restriction. Effects on blood pressure and renal hemodynamics in hypertensive patients. JAMA 142; 711-715.

Chonko AM, Grantham JJ (1981).
Factors that affect the renal handling of urate in man. In: The Kidney. BM Brenner, FC Rector (Eds). WB Saunders company, Philadelphia, London, Toronto, pp 1039-1042.

Cleland JGF, Dargie HJ, McAlpine H, Ball SG, Morton JJ, Robertson JIS, Ford I (1985).
Severe hypotension after first dose of enalapril in heart failure. Br Med J 291, 1309-1312.

Cohen ML, Kurz KD, Wiley Schenck K (1983).
Tissue angiotensin converting enzyme inhibition as an index of the disposition of enalapril (MK 421) and metabolite MK 422. J Pharmacol Exp Ther 226; 192-196.

Cooper RA (1983).
Captopril-associated neutropenia: who is at risk ? Arch Intern Med 143; 659-660.

Cushman DW, Cheung HS, Sabo EF, Ondetti MA (1981).
Angiotensin converting enzyme inhibitors: evolution of a new class of antihypertensive drugs. In: Angiotensin converting enzyme inhibitors. Mechanism of action and clinical implications. ZP Horowitz (Ed). Urban and Schwarzenberg, Baltimore-Munich, pp 3-27.

Davidow M, Gavrilovich L, Mroczek W, Finnerty FA (1969).
Relation of extracellular fluid volume to arterial pressure during drug-induced saluresis. Circulation 40; 349-355.

Davis JO (1961).
Mechanisms regulating the secretion and metabolism of aldosterone in experimental secondary hyperaldosteronism. Recent Prog Horm Res 17; 293-352.

Davis JO, Freeman RH (1976).
Mechanisms regulating renin release. Physiol Rev 56; 1-56.

De Bono E, De J, Lee F R et al (1963).
The action of angiotensin in man. Clin Sci 25; 123-157.

DiPette DJ, Ferraro JC, Evans RR, Martin M (1985).
Enalaprilat, an intravenous angiotensin converting enzyme inhibitor, in hypertensive crises. Clin Pharmacol Ther 38; 199-204.

Dollery CT (1981).
Does it matter how blood pressure is reduced? Clin Sci 61; 443s-445s.

Dollery CT (1984).
Management of hypertension: risk benefit ratio. J Hypertens 2, Supp 2; 9-12.

Donker AJM, van der Hem GK, Sluiter WJ, Beekhuis H (1977).
A radio-isotope method for simultaneous determination of the glomerular filtration rate and the effective renal plasma flow. Neth J Med 20; 97-103.

Dustan HP, Schneckloth RE, Corcoran AC, Page IH (1958).
The effectiveness of long-term treatment of malignant hypertension. Circulation 18; 644-651.

Dustan HP, Tarazi RC, Bravo EL (1974).
Diuretic and diet treatment of hypertension. Arch Int Med 133; 1007-1013.

Dustan HP (1983).
Causes of inadequate response to antihypertensive drugs. Volume factors. Hypertension 5, supp III; 26-31.

Dyckner T, Wester PO (1983).
Effect of magnesium on blood pressure. Br Med J 286; 1847-1849.

Editorial (1980).
Captopril: benefits and risks in severe hypertension. Lancet ii; 129-130.

Edwards RM (1983).
Segmental effects of norepinephrine and angiotensin on isolated renal microvessels. Am J Physiol 244; F526-534.

Enalapril in hypertension study group (1984).
Enalapril in essential hypertension: a comparative study with propranolol. Br J Clin Pharmacol 18; 51-56.

Ferguson RK, Irvin JD, Swanson BN, Lee RB (1982).
A comparative pilot study of enalapril, a new angiotensin converting enzyme inhibitor and hydrochlorothiazide in essential hypertension. J Clin Pharmacol 22; 281-289.

Ferguson RK, Irvin JD, Swanson BN, Vlasses PH, Bergquist PA, Till AE (1983).
Food does not alter the absorption of enalapril maleate (MK 421) as measured by the disposition of its active metabolite (MK 422). Clin Pharmacol Ther 33; 254.

Finnerty FA (1962).
Hemodynamics of angiotensin in man. Circulation 25; 255-258.

Finnerty FA jr, Davidow M, Kalaviatos N (1968).
Relation of sodium balance to arterial pressure during drug-induced saluresis. Circulation 37; 175-183.

Fouad FM, Tarazi RC, Bravo EL, Textor SC (1984).
Hemodynamic and antihypertensive effects of the new oral angiotensin converting enzyme inhibitor MK 421 (enalapril). Hypertension 6; 167-174.

Freedlander AE, Fyhrquist F, Hollemans HJG (1974).
In: Methods of hormone radio-immuno assay . BM Jaffe, HR Behrmann (Eds), New York, London, Academic press, pp 445.

Freis ED (1979).
Salt in hypertension and the effects of diuretics. Ann Rev Pharmacol Toxicol 19; 13-23.

Gavras H, Biollaz J, Waeber B, Brunner HR, Gavras I, Davies RO (1981).
Antihypertensive effect of the new oral angiotensin converting enzyme inhibitor "MK 421". Lancet ii; 543-546.

Genest J, Nowaczynski W, Koiw E, Sandor T, Biron P (1960).
Adrenocortical function in essential hypertension. In: Essential hypertension. KD Bock, PT Cottier (Eds). Berlin, Springer Verlag, pp126-146.

Gezondheidsraad (1983).
Advies inzake hypertensie. Min WVC, Verslagen, adviezen, rapporten 1983, no 4. Staatsuitgeverij, 's Gravenhage.

Giese J, Rasmussen S, Damkjaer Nielsen M, Ibsen H (1983).
Biochemical monitoring of vasoactive peptides during angiotensin converting enzyme inhibition. J Hypertension 1, supp 1; 31-36.

Gifford RW jr (1984).
The role of diuretics in the treatment of hypertension. Am J Med 77 (4A); 102-109.

Given BD, Taylor T, Hollenberg NK, Williams GH (1984).
Duration of action and short-term hormonal responses to enalapril (MK 421) in normal subjects. J Cardiovasc Pharmacol 6; 436-441.

Glück Z, Beretta-Piccoli C, Reubi FC (1984).
Long-term effects of captopril on renal function in hypertensive patients. Eur J Clin Pharmacol 26; 314-323.

Goldblatt H, Lynch J, Hanzal RF, Summerville WW (1934).
Studies on experimental hypertension.1. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J Exp Med 59; 347-380.

Good DW, Wright FS (1979).
Luminal influences on potassium secretion: sodium concentration and fluid flow rate. Am J Physiol 236; F192-205.

Gross F (1958).
Renin und Hypertensin, physiologische oder pathologische Wirkstoffe? Klin Wochenschr 36; 693-706.

Gross DM, Sweet CS, Ulm EH et al (1981).
Effect of N-[(S)-1-carboxy-3-phenylpropyl]-L-ala-L-pro and its ethyl ester (MK 421) on angiotensin converting enzyme in vitro and angiotensin I pressor responses in vivo. J Pharmacol Exp Ther 16; 552-557.

Guyton AC, Coleman TG, Cowley AW jr, Manning RD jr, Norman RA jr, Ferguson JD (1974)
A systems analysis approach to understanding long-range arterial blood pressure control and hypertension. Circ Res 35; 159-176.

Haber E (1983).
Inhibitors of renin: present and future. Clin Exp Hypertens A5; 1193-1205.

Hall JE, Guyton AC, Smith MJ, Coleman TG (1980).
Blood pressure and renal function during chronic changes in sodium intake: role of angiotensin. Am J Physiol 239; F271-280.

Hamilton M, Pickering GW, Roberts JAF, Sowry GSC (1954).
The aetiology of essential hypertension.1. The arterial pressure in the general population. Clin Sci 13; 11-35.

Harrington JT, Isner JM, Kassirer JP (1982).
Our national obsession with potassium. Am J Med 73; 155-159.

Harris PJ, Navar LG, Ploth DW (1984).
Evidence for angiotensin stimulated proximal tubular fluid reabsorption in normotensive and hypertensive rats: effect of acute administration of captopril. Clin Sci 66; 541-544.

Harris PJ, Navar LG (1985).
Tubular transport responses to angiotensin. Am J Physiol 248 (Renal fluid electrolyte physiol 17); F 621-630.

Haynes RB, Taylor DW, Sackett DL (1983).
Hypertension in the real world. In: Hypertension: pathophysiology and treatment. J Genest, O Kuchel, P Hamet, M Cantin (Eds). New York, McGraw-Hill, pp 1251-1261.

Helgeland A (1980).
Treatment of mild hypertension: a five year controlled drug trial. The Oslo Study. Am J Med 69; 725-732.

Hesse B, Nielsen I, Lund-Jacobsen H (1975).
The early effects of intravenous furosemide on central hemodynamics, venous tone and plasma renin activity. Clin Sci 49; 551-555.

Hodsman GP, Isles CG, Murray GD, Usherwood TP, Webb DJ, Robertson JIS (1983).
Factors related to the first dose hypotensive effect of captopril: prediction and treatment. Br Med J 286; 832-834.

Hodsman GP, Zabludowski JR, Zoccali C, Fraser R, Morton JJ, Murray D, Robertson JIS (1984).
Enalapril (MK 421) and its lysine analogue (MK 521): a comparison of acute and chronic effects on blood pressure, renin angiotensin system and sodium excretion in normal man. Br J Clin Pharmacol 7; 233-241.

Hollenberg NK, Adams DF, Solomon HS, Rashid A, Abrams HL, Merrill P (1974a).
Senescence and the renal vasculature in normal man. Circ Res 34; 309-319.

Hollenberg NK, Chenitz WR, Adams DF, Williams GH (1974b).
Reciprocal influence of salt intake on adrenal glomerulosa and renal vascular responses to angiotensin II in normal man. J Clin Invest 54; 34-52.

Hollenberg NK, Williams GH, Taub KJ, Ishikawa I, Brown C, Adams F (1977).
Renal vascular response to interruption of the renin angiotensin system in normal man. Kidney Int 12; 285-293.

Hollenberg NK, Swartz SL, Passan DR, Williams GH (1979).
Increased glomerular filtration rate after converting enzyme inhibition. N Engl J Med 301; 9-12.

Hollenberg NK, Meggs LG, Williams GH, Katz J, Garnic JD, Harrington DP (1981).
Sodium intake and renal responses to captopril in normal man and in essential hypertension. Kidney Int 20; 240-245.

Hoorntje SJ, Weening JJ, The TH, Kallenberg CGM, Donker AJM, Hoedemaeker PJ (1980).
Immune-complex glomerulopathy in patient treated with captopril. Lancet i; 1212-1214.

Hoorntje SJ (1981).
Merits and demerits of the converting enzyme inhibitor captopril in antihypertensive treatment. Thesis, Groningen.

Hypertension Detection and Follow-up Program Cooperative Group (1979).
Five year findings of the Hypertension Detection and Follow-up Program I. Reduction in mortality in persons with high blood pressure including mild hypertension. II. Mortality by race, sex and age. JAMA 242; 2562-2572.

Ibsen H, Egan B, Osterziel K, Vander A, Julius S (1983).
Reflex-hemodynamic adjustments and baroreflex sensitivity during converting enzyme inhibition with MK 421 in normal humans. Hypertension 5, supp I; 184-191.

IPPPSH Collaborative Group (1985).
Cardiovascular risk and risk factors in a randomized trial of treatment based on the beta blocker oxprenolol. The International Prospective Primary Prevention Study in Hypertension. J Hypertension 3; 379-392.

Janeway TC (1913).
A clinical study of hypertensive cardiovascular disease. Arch Intern Med 12; 755.

Johns DW, Baker KM, Ayers CR et al (1980).
Acute and chronic effects of captopril in hypertensive patients. Hypertension 2; 567-575.

Johnson MD, Malvin RL (1977).
Stimulation of renal sodium reabsorption by angiotensin II. Am J Physiol, 232 (4): F 298-306.

Johnston CI, Jackson B, McGrath B, Matthews G, Arnolda L (1983).
Relationship of antihypertensive effect of enalapril to serum MK 42 levels and angiotensin converting enzyme inhibition. J Hypertension 1, supp 1; 71-77.

Johnston CI, Jackson B, Cubela R, Arnolda L (1984).
Mechanism for hypotensive action of angiotensin converting enzyme inhibitors. Clin Exp Hypertens A 6 (1&2); 551-561.

Kallenberg CGM, van der Laan S, de Zeeuw D (1981).
Captopril and the immune system. Lancet ii; 92.

Kaplan NM (1984).
Our appropriate concern about hypokalemia. Am J Med 77; 1-4.

Kawasaki T, Delea CS, Bartter FC, Smith H (1978).
The effect of high sodium and low sodium intakes on blood pressure and other related variables in human subjects with idiopathic hypertension. Am J Med 64; 193-198.

Keeton TK, Campbell WB (1981).
The pharmacological alteration of renin release. Pharmacol Rev 1; 81-227.

Kelly RA, Wilcox CS, Mitch WE et al (1983).
Response of the kidney to furosemide. II. Effect of captopril on sodium balance. Kidney Int 24; 233-239.

Kimbrough HM jr, Vaughan DE jr, Carey RM, Ayers CR (1977).
Effect of intrarenal angiotensin II blockade on renal function in conscious dogs. Circ Res 40; 174-178.

Kohlstaed KG, Helmer OM, Page IH (1938).
Activation of renin by blood colloids. Proc Soc Exp Med Biol 39; 214-215.

Langford HG (1983).
Dietary potassium and hypertension: epidemiologic data. Ann Intern Med 98; 770-772.

Laragh JH, Angers M, Kelly WG, Lieberman S (1960).
Hypotensive agents and pressor substances. The effect of epinephrine, norepinephrine and angiotensin II, and others on the secretory rate of aldosterone in man. JAMA 174; 234-240.

de Leeuw PW, Hoogma RPLM, van Soest GAW, Tchang PT, Birkenhäger WH (1983)
Humoral and renal effects of MK 421 (enalapril) in hypertensive subjects. J Cardiovasc Pharmacol 5; 731-736.

Lieberman J, Sastre A (1983).
Angiotensin converting enzyme activity in post-mortem human tissue. Lab Invest 48; 711-717.

Luft FC, Weinberger MH, Grim CE (1982).
Sodium sensitivity and resistance in normotensive humans. Am J Med 72; 726-736.

Lund-Johansen P, Omvik P (1984).
Long-term hemodynamic effects of enalapril (alone and in combination with hydrochlorothiazide) at rest and during exercise in essential hypertension. J Hypertension 2, supp 2; 49-56.

MacGregor GA, Markandu ND, Roulston JE, Jones JC, Morton JJ (1981).
Maintenance of blood pressure by the renin angiotensin system in normal man. Nature 291; 329-331.

MacGregor GA, Markandu ND, Smith SJ, Sagnella GA, Morton JJ (1983a).
Angiotensin converting enzyme inhibition reveals an important role for the renin system in the control of normal and high blood pressure in man. Clin Exp Hypertens A 5 (7&8); 1367-1380.

MacGregor GA, Smith SJ, Markandu ND, Banks RA, Sagnella GA (1983b).
Moderate potassium supplementation in essential hypertension. Lancet ii; 567-570.

Man in't Veld AJ, Schicht IM, Derkx FHM, de Bruyn JHB, Schalekamp MADH (1980).
Effects of an angiotensin converting enzyme inhibitor (captopril) on blood pressure in anephric subjects. Br Med J 280; 288-290.

McCaa RE, Hall JE, McCaa CS (1978).
The effects of angiotensin I converting enzyme inhibition on arterial blood pressure and urinary sodium excretion. Role of the renal renin angiotensin and kallikrein-kinin system. Circ Res 43, supp 1; 32-39.

McCarron DA, Morris CD, Cole C (1982).
Dietary calcium in human hypertension. Science 217; 267-269.

McFate Smith W, Kulaga SF, Moncloa F, Pingeon R, Walker JF (1984).
Overall tolerance and safety of enalapril. J Hypertension 2, supp 2; 113-117.

McNabb WR, Noormohamed FH, Brooks BA, Till AE, Lant AF (1985).
Effects of repeated doses of enalapril on renal function in man. Br J Clin Pharmacol 19; 353-361.

Medical Research Council Working Party (1981).
Adverse reactions to bendrofluazide and propranolol for the treatment of mild hypertension. Lancet ii; 539-543.

Medical Research Council Working Party (1985).
MRC trial of treatment of mild hypertension; principal results. Br. Med J 291; 97-104.

Meggs LG, Hollenberg NK (1980).
Converting enzyme inhibition and the kidney. Hypertension 2; 551-557.

Millar JA, Derkx FHM, McLean K, Reid JL (1982).
Pharmacodynamics of converting enzyme inhibition: the cardiovascular, endocrine and autonome reflexes of MK 421 (enalapril) and MK 521. Br. J Clin Pharmacol 14; 347-355.

Mimran A, Guiod L, Hollenberg NK (1974).
The role of angiotensin in the renal response to salt restriction. Kidney Int 5; 348-355.

Miyazaki M, Okunishi H, Nishimura K, Toda N (1984).
Vascular angiotensin converting enzyme actvity in man and other species. Clin Sci 66; 39-45.

Moore TJ, Crantz FR, Hollenberg NK et al (1981).
Contributions of prostaglandins to the antihypertensive action of captopril in essential hypertension. Hypertension 3; 168-173.

Morris DJ (1981).
The metabolism and mechanism of action of aldosterone. Endocr Rev 2; 234-247.

Muirhead EE (1980).
Antihypertensive functions of the kidney. Hypertension 2; 444-464.

Multiple Risk Factor Intervention Trial research group (1982).
Multiple risk factor intervention trial. Risk factor changes and mortality results. JAMA 248; 1465-1477.

Navar LG, Langford HG (1974).
Effects of angiotensin on the renal circulation. In: Angiotensin. Handbook of experimental pharmacology 37. IH Page, MF Bumpus (Eds). Berlin, New York, Springer Verlag, pp 455-474.

Navis GJ, de Jong PE, Kallenberg CGM, de Monchy JGR, de Zeeuw D (1984).
Absence of cross-reactivity between captopril and enalapril. Lancet i; 1017.

Nüssberger J, Brunner DB, Waeber B, Brunner HR (1985).
True versus immunoreactive angiotensin II in human plasma. Hypertension 7, supp I; 1-7.

Oelkers W, Brown JJ, Fraser R, Lever AF, Morton JJ, Robertson JIS (1974).
Sensitization of the adrenal cortex to angiotensin II in sodium-deplete man. Circ Res 34; 69-77.

Omvik P, Tarazi RC, Bravo EL (1980).
Regulation of sodium balance in hypertension. Hypertension 2; 515-523.

Ondetti MA, Rubin B, Cushman DW (1977).
Design of specific inhibitors of angiotensin converting enzyme: new class of orally active antihypertensive agents. Science 196; 441-444.

Ondetti MA, Cushman DW (1982).
Enzymes of the renin-angiotensin system and their inhibitors. Ann Rev Biochem 51; 283-308.

Oparil S, Tregear GW, Koerner TJ, Barnes BA, Haber E (1971).
Mechanism of pulmonary conversion of angiotensin I to II in the dog. Circ Res 29; 682-690.

Page IH, Helmer OM (1940).
A crystalline pressor substance (angiotonin) resulting from the action between renin and renin activator. J Exp Med 71; 29-42.

Patchett AA, Harris E, Tristam EW et al (1980).
A new class of angiotensin converting enzyme inhibitors. Nature 88; 280-282.

Paul M, Hermann K, Printz M, Lang RE, Unger T, Ganten D (1983).
The brain angiotensin system: subcellular localisation and interferences with converting enzyme inhibitors. J Hypertension 1, supp 1; 9-15.

Peach MJ (1977).
Renin angiotensin system: biochemistry and mechanism of action. Physiol Rev 57; 313-370.

Peart WS (1983).
General review of hypertension. In: Hypertension. Physiopathology and treatment. J Genest, O Kuchel, P Hamet, M Cantin (Eds). New York, McGraw-Hill. pp 3-12.

Pickering GW (1955).
High blood pressure. London, J&A Churchill Ltd, pp 316-317.

Pratt JJ, Boonman R, Woldring MG, Donker AJM (1978).
Special problems in radio-immuno assay of plasma aldosterone without prior extraction and purification. Clin Chim Acta 84; 329-337.

Prins EJL (1979a).
Nephrotic syndrome in patient on captopril. Lancet ii; 306.

Prins EJL(1979b).
Converting-enzyme inhibition, experiences with captopril in hypertensive patients. Thesis, Groningen.

Reid WD, Laragh JH (1965).
Sodium and potassium intake, blood pressure and pressor response to angiotensin. Proc Soc Exp Biol Med 120; 26-29.

Reubi FC, Vorburger C, Bütikofer E (1970).
A comparison of the short-term and long-term hemodynamic effects of antihypertensive drug therapy. In: Catapres in hypertension. Connolly ME (Ed). London, Butterworth, pp 113-116.

Reubi FC (1978).
Role of physical factors in the acute changes in renal function elicited by antihypertensive drugs. Eur J Clin Pharmacol 13; 185-190.

Rittel W, Iselin B, Kappeller H, Riniker B, Schwyzer R (1957).
Synthese eines hochwirksamen Hypertensin II-amids. Helv Chim Acta 40; 614-624.

Robertson PW, Klidjian A, Harding LK, Walters G, Lee MR, Robb-Smith AHT (1967).
Hypertension due to a renin-secreting renal tumour. Am J Med 43; 963-976.

Romero JC, Staneloni RJ, Dufau ML, Dohmen R, Binia A, Kliman B, Fasciolo JC (1968).
Changes in fluid compartments, renal hemodynamics, plasma renin and aldosterone secretion induced by low sodium intake. Metabolism 17; 10-19.

Rubin B, Antonaccio MJ (1980).
Captopril. In: Pharmacology of antihypertensive drugs. A Scriabin (Ed). New York, Raven Press, pp 21-42.

Sambhi MP (1983).
Prorenin and activators and inhibitors of renin. In: Hypertension. Pathophysiology and treatment. J Genest, O Kuchel, P Hamet, M Cantin (Eds). New York, McGraw-Hill, pp 210-224.

Saris S, Lowenthal D, Klein L, Irvin JD, Till AE, Hichens M, Harris KE (1984).
Enalapril maleate in renal failure. Clin Pharmacol Ther 35; 272.

Schalekamp MADH, Schalekamp-Kuyken MPA, Birkenhäger WH (1970).
Abnormal renal hemodynamics and renin suppression in hypertensive patients. Clin Sci 38; 101-110.

Schuster VL, Kokko JP, Jacobson HR (1984).
Angiotensin directly stimulates sodium transport in rabbit proximal convoluted tubules. J Clin Invest 73; 507-515.

Schwarz H, Bumpus FM, Page IH (1957).
Synthesis of a biologically active peptide similar to natural isoleucine angiotonin octapeptide. J Am Chem Soc 79; 5697-5703.

Seldin DW (1973).
Evaluation of clearance methods for localization of site of action of diuretics. In: Modern diuretic therapy in the treatment of cardiovascular and renal disease. AF Lant, GM Wilson (Eds). Amsterdam, Exerpta Medica, pp 97-111.

Selkurt EE (1951).
Effects of pulse pressure and mean arterial pressure modifications on renal hemodynamics and electrolyte and water excretion. Circulation 4; 541-551.

Severs WB, Daniels-Severs AE (1973).
Effects of angiotensin on the central nervous system. Pharmacol Rev 25; 415-449.

Shipley RE, Study RE (1951).
Changes in renal blood flow, extraction of inulin, glomerular filtration rate and urine flow with acute alterations of renal artery pressure. Am J Physiol 167; 676-688.

Shurtleff D (1973).
Some characteristics related to the incidence of cardiovascular disease and death: Framingham Study, 18-year follow-up. In: The Framingham study: an epidemiological investigation of cardiovascular disease, section 30. WB Kannel, T Gordon (Eds). Dept of HEW publication, Washington DC (NIH) 74-599.

Silas JH, Barker AT, Ramsay LE (1980).
Clinical evaluation of Dinamap 845 automated blood pressure recorder. Br Heart J 43; 202-205.

Simon S, Morioka S, Snyder DK, Cohn JN (1983).
Increased renal plasma flow in long-term enalapril treatment of hypertension. Clin Pharmacol Ther 34; 459-465.

Skeggs LT jr, Marsh WH, Kahn JR, Shumway NP (1954).
The existence of two forms of hypertensin. J Exp Med 99; 275-282.

Society of Actuaries (1959).
Build and Blood Pressure Study, Chicago, Society of Actuaries.

Steinhausen M, Snoei H, Parekh N, Baker R, Johnson PC (1983).
Hydronephrosis: a new method to visualize vas afferens, efferens and glomerular network. Kidney Int 23; 794-806.

Strewler GJ, Hinrichs KJ, Guiod LR, Hollenberg NK (1972).
Sodium intake and vascular smooth muscle responsiveness to norepinephrine and angiotensin in the rabbit. Circ Res 31; 758-766.

Struyker-Boudier HAJ (1980).
Dynamic systems analysis as a basis for drug design: application to antihypertensive drug action. In: Drug Design, Vol X, New York, London, Academic Press, pp 145-191.

Studer A, Vetter W (1982).
Reversible leucopenia associated with angiotensin converting enzyme inhibitor MK 421. Lancet i; 458.

Swartz SL, Williams GH, Hollenberg NK, Moore TJ, Dluhy RG (1979).
Converting enzyme inhibition in essential hypertension: the hypotensive response does not only reflect reduced angiotensin II formation. Hypertension 1; 106-111.

Swartz SL, Williams GH, Hollenberg NK, Levine L, Dluhy RG, Moore TJ (1980).
Captopril induced changes in prostaglandin production. Relationship to vascular responses in man. J Clin Invest 65; 1257-1264.

Sweet CS, Arbegast PT, Gaul SL, Blaine EH, Gross DM (1981).
Relationship between angiotensin I blockade and antihypertensive properties of single doses of MK 421 and captopril in spontaneous and renal hypertensive rats. Eur J Pharmacol 76; 167-176.

Sweet CS (1983).
Pharmacological properties of the converting enzyme inhibitor enalapril maleate (MK 421). Fed Proc 42; 167-170.

Tarazi RC, Bravo EL, Fouad FM, Omvik P, Cody RJ (1980).
Hemodynamic and volume changes with captopril. Hypertension 2; 576-585.

Tarazi RC (1985).
The second step in "stepped-care": addition or substitution? J Cardiovasc Pharmacol 7; S121-S125.

Thomsen K (1984).
Lithium clearance: a new method for determining proximal and distal tubular reabsorption of sodium and water. Nephron 37; 217-221.

Thurston H, Swales JD (1977).
Blood pressure response of nephrectomized hypertensive rats to converting enzyme inhibitor: evidence for persisting vascular renin activity. Clin Sci 53; 299-304.

Tigersted R, Bergman PG (1898).
Niere und Kreislauf. Skand Arch Physiol 8; 223-271.

Ulm EH (1983).
Enalapril maleate (MK 421), a potent nonsulfhydryl angiotensin converting enzyme inhibitor; absorption, disposition and metabolism in man. Drug Metab Rev 14; 99-110.

Unger TE, Schull B, Rascher W, Lang RE, Ganten D (1982).
Selective activation of the converting enzyme inhibitor MK 421 and comparison of its active diacid form with captopril in different tissues of the rat. Biochem Pharmacol 31; 3063-3070.

Vander AJ, Carlson J (1969).
Mechanisms of the effects of furosemide on renin secretion in anesthetized dogs. Circ Res 25;145-152.

Velasquez MT, Notargiacomo AV, Cohn JN (1972).
Influence of cortical plasma transit time on p-aminohippurate extraction during induced renal vasodilation in anaesthetized dogs. Clin Sci 43; 401-411.

Veterans Administration Cooperative Study Group on Antihypertensive Agents (1967).
Effects of treatment on morbidity and mortality. I. Results in patients with diastolic blood pressure averaging 115 through 129 mmHg. JAMA 202; 1028-1038.

Veterans Administration Cooperative Study Group on Antihypertensive Agents (1970).
Effects of treatment on morbidity and mortality. II. Results in patients with diastolic blood pressure averaging 90 through 114 mmHg. JAMA 213; 1143-1152.

Veterans Administration Cooperative Study Group on Antihypertensive Agents (1983).
Low-dose captopril for the treatment of mild to moderate hypertension. Hypertension 5, supp III; 139-144.

Vidt DG, Bravo EL, Fouad FM (1982).
Drug therapy: captopril. N Engl J Med 306; 214-219.

Waeber B, Brunner HR, Brunner DB, Curtet A-L, Turini G, Gavras H (1980).
Discrepancy between antihypertensive effect and angiotensin converting enzyme inhibition by captopril. Hypertension 2; 236-242.

ter Wee PM, Smit AJ, Rosman JB, Sluiter WE, Donker AJM (1986).
The effect of intravenous infusion of a low dose dopamine on renal function in normal individuals and in patients with renal disease. Am J Nephrol 6; 42-46.

Wenting GJ, Tan-Tjiong HL, Derkx FHM, de Bruyn JHB, Man in't Veld AJ, Schalekamp MADH (1984).
Split renal function after captopril in unilateral renal artery stenosis. Br Med J 288; 886-890.

Wesson LG jr (1964).
Electrolyte excretion in relation to diurnal cycles of renal function. Medicine 43; 547-592.

Whelton PK (1984).
Essential hypertension. Therapeutic implications of epidemiological risk estimation. J Hypertension 2, supp 2; 3-8.

WHO (1983).
Guidelines for the treatment of mild hypertension: memorandum from a WHO/ISH meeting. Lancet i; 457-458.

Zanchetti A (1985).
A re-examination of stepped-care: a retrospective and a prospective. J Cardiovasc Pharmacol 7; S126-S131.

Zimmerman BG (1978).
Actions of angiotensin on adrenergic nerve endings. Fed Proc 37; 199-202.

Zimmerman BG, Wong PC (1981).
Alterations in renal function produced by angiotensin converting enzyme inhibitors. In: Angiotensin converting enzyme inhibitors. Mechanisms of action and therapeutic implications. ZP Horowitz (Ed). Baltimore, Munich, Urban&Schwarzenberg, pp 239-255.

Zimmerman BG, Finis Carla J (1985).
Comparison of systemic and intrarenal converting enzyme inhibition by MK-422 on renal hemodynamics in conscious dogs. J Cardiovasc Pharmacol 7; 715-722.

Zusman RM (1984).
Renin- and non-renin-mediated antihypertensive actions of converting enzyme inhibitors. Kidney Int 25; 969-983.